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PyroMark® Q96 ID User Manual

QIAGEN®

Sample & Assay Technologies

Trademarks

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1 Safety Information

Before using the PyroMark Q96 ID, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

The following types of safety information appear throughout this manual.

WARNING	The term WARNING is used to inform you about situations that could result in **personal injury** to you or other persons. Details about these circumstances are given in a box like this one.

CAUTION	The term CAUTION is used to inform you about situations that could result in **damage to the instrument** or other equipment. Details about these circumstances are given in a box like this one.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

1.1 Proper use

WARNING/ CAUTION	**Risk of personal injury and material damage** [W1] Improper use of the PyroMark Q96 ID may cause personal injuries or damage to the instrument. The PyroMark Q96 ID must only be operated by qualified personnel who have been appropriately trained. Servicing of the PyroMark Q96 ID must only be performed by QIAGEN Field Service Specialists.

WARNING/ CAUTION	**Risk of personal injury and material damage** [W2] The PyroMark Q96 ID is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

Perform the maintenance as described in Section 7. QIAGEN charges for repairs that are required due to incorrect maintenance.

1.2 Electrical safety

Disconnect the line power cord from the power outlet before servicing.

WARNING	**Electrical hazard** [W3] Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited. **Lethal voltages inside the instrument** When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.

To ensure satisfactory and safe operation of the PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation, follow the advice below:

- The line power cord must be connected to a line power outlet that has a protective conductor (earth/ground)
- Keep mains plugs easily accessible in case the equipment needs to be disconnected quickly from mains power
- Use only power supplies and cords supplied with the instrument
- When replacing the mains fuse, replace only with the type and current rating specified on the rating label

1.3 Chemical hazards

WARNING	**Hazardous chemicals** [W4] The Denaturation Solution used with the Vacuum Workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA*, ACGIH†, or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

1.4 Mechanical hazards

The lid of the PyroMark Q96 ID must remain closed during operation of the instrument.

WARNING	**Moving parts** [W5] To avoid contact with moving parts during operation of the PyroMark Q96 ID, the instrument must be operated with the lid closed. Do not remove the cover panels since there are no user-serviceable parts inside. If there is a problem with the PyroMark Q96 ID, contact QIAGEN Technical Services immediately.

WARNING	**Pinch and impact hazards** [W6] The dispensing unit, process chamber lid, and robot may move at any time.

WARNING	**Sharp needles** [W7] Do not touch the sharp needles at the bottom of the reagent cartridge. Handle the needles with care. Small particles and fibers may obstruct the needles.

1.5 Heat hazards

WARNING	**Hot surface** [W8] The external heating block can reach temperatures of up to 80°C (176°F). Avoid touching them when they are hot.

CAUTION	**Risk of overheating** [C1] To ensure proper ventilation, maintain a minimum clearance of 10 cm (4 in.) at the sides and rear of the PyroMark Q96 ID. Slits and openings that ensure the ventilation of the PyroMark Q96 ID must not be covered.

1.6 Symbols used on the PyroMark Q96 ID

Symbol	Location	Language	Description
	Type plate on the back of the instrument	EN	CE mark
	All products	EN	Catalog or recorder number
	All products	EN	Temperature limitation
	Type plate on the back of the instrument and all other products	EN	Legal manufacturer

Symbol	Location	Language	Description
	Type plate on the back of the instrument	EN	Warning, consult user manual
	Type plate on the back of the instrument and box	EN	Serial number
	Type plate on the back of the instrument	EN	Waste Electrical and Electronic Equipment (WEEE)
	Type plate on the back of the instrument	EN	FCC mark of the United States Federal Communications Commission
	Type plate on the back of the instrument	EN	C-Tick mark for Australia (supplier identification N17965/N15128)
	Type plate on the back of the instrument	EN	RoHS mark for China (the restriction of the use of certain hazardous substances in electrical and electronic equipment)
	Type plate on the back of the instrument	EN	CSA listing mark for Canada and the USA

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2 Introduction

Thank you for choosing the PyroMark Q96 ID. We are confident it will become an integral part of your laboratory.

Before using the PyroMark Q96 ID, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition. A French and German translation of the safety information can be found in Appendices D and E, respectively.

2.1 About this user manual

This user manual provides information about the PyroMark Q96 ID in the following sections:

1. Safety Information
2. Introduction
3. General Description
4. Installation Procedures
5. Operating Procedures
6. PyroMark Q96 Software
7. Maintenance
8. Troubleshooting
9. Glossary

   Appendices

The appendices include:

- Technical data
- Environmental conditions
- WEEE recycling requirements
- Assay design and validation
- Warranty terms
- French and German translations of safety information

2.2 General information

2.2.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding the PyroMark Q96 ID or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

2.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

2.2.3 Version management

This document is the *PyroMark Q96 ID User Manual*, version 4.0.

2.3 Intended use of the PyroMark Q96 ID

The PyroMark Q96 ID System detects changes in specified variable positions in, or base-calling of, DNA prepared from biological samples.

The PyroMark Q96 ID Instrument and PyroMark Q96 Vacuum Workstation are intended to be used only in combination with QIAGEN kits indicated for use with the PyroMark Q96 ID Instrument for the applications described in the kit handbooks.

If the PyroMark Q96 ID Instrument and PyroMark Q96 Vacuum Workstation are used with kits other than QIAGEN kits, it is the user's responsibility to validate the performance of such product combination for any particular application.

The PyroMark Q96 ID System is intended for use by professional users, such as technicians and physicians trained in molecular biological techniques and the operation of the PyroMark Q96 ID System.

2.3.1 Requirements for PyroMark Q96 ID users

The table below covers the general level of competence and training necessary for transportation, installation, use, maintenance, and servicing of the PyroMark Q96 ID.

Task	Personnel	Training and experience
Delivery	No special requirements	No special requirements
Installation	**QIAGEN Field Service Specialists only**	
Routine use (running protocols)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Preventive maintenance	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Servicing and annual preventive maintenance	**QIAGEN Field Service Specialists only**	

3 General Description

The PyroMark Q96 ID is a multi-application sequence analysis system which uses Pyrosequencing® technology for the real-time analysis of specific DNA sequences, including single nucleotide polymorphisms (SNPs), mutation, and methylation analysis. The instrument can analyze 96 samples simultaneously. An easy-to-use protocol is used to prepare samples.

Enclosed in the light-proof instrument housing, a light detection system with cooled charged coupled device (CCD) camera detects light emitted from reactions in the wells of the PyroMark Q96 Plate Low. The instrument includes an embedded computer module with hard disk and network interface.

3.1 PyroMark Q96 ID definitions

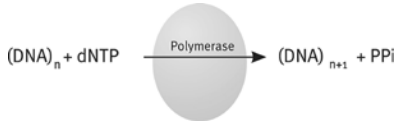
- PyroMark Q96 ID Instrument: Instrument only
- PyroMark Q96 Software: Software only
- PyroMark Q96 Vacuum Workstation: Vacuum Workstation only
- PyroMark Q96 ID: Instrument, software, and installation
- PyroMark Q96 ID System: All of the above, plus any PyroMark kits

3.2 Pyrosequencing principle

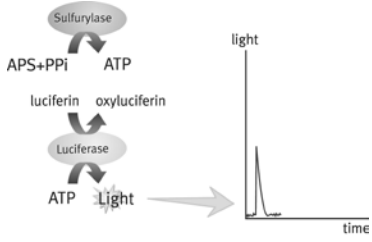
Pyrosequencing uses sequencing by synthesis for accurate and quantitative analysis of DNA sequences.

1. A sequencing primer is hybridized to a single-stranded, PCR-amplified DNA template.
2. The template is incubated with enzymes and substrates.
3. The first of four nucleotides is added to the reaction. If the nucleotide is complementary to the base in the template strand it will be incorporated into the DNA strand by the DNA polymerase.

4. Each incorporation event is accompanied by release of pyrophosphate (PPi) in an equimolar quantity to the amount of nucleotide incorporated.



5. ATP sulfurylase quantitatively converts PPi to ATP in the presence of adenosine 5' phosphosulfate.
6. This drives the conversion of luciferin to oxyluciferin by luciferase, generating visible light in amounts proportional to the amount of ATP. Light is detected using charged coupled devices (CCDs) and seen as a peak in the Pyrogram®. Each light signal is proportional to the number of nucleotides incorporated.

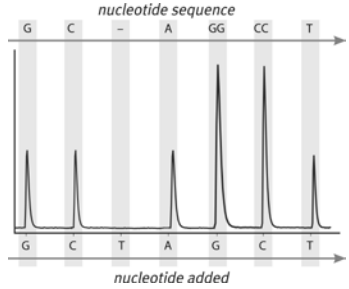


nucleotide incorporation generates light seen as a peak in Pyrogram

7. Apyrase, a nucleotide-degrading enzyme, continuously degrades unincorporated nucleotides and ATP. When degradation is complete, another nucleotide is added.

8. Nucleotides are added one at a time.
   Note: Deoxyadenosine alfa-thio triphosphate (dATPαS) is used instead of natural deoxyadenosine triphosphate (dATP) since it is used efficiently by the DNA polymerase, but not recognized by the luciferase.

9. As the process continues, the complementary sequence is built up and the nucleotide sequence is determined from the peak in the Pyrogram.



3.3 PyroMark Q96 ID principle

The PyroMark Q96 ID performs DNA sequencing using Pyrosequencing technology.

1. After setting up the run in the PyroMark Q96 Software, the PyroMark Q96 Plate Low containing DNA samples is placed on the heating block in the instrument and the reagent cartridge containing enzymes, substrate, and nucleotides is placed in the dispensing unit.
2. The run is then started when the user clicks the “Run” button in the “Run Setup” dialog box. The operator’s computer sends all information required for the analysis to the instrument.

   Note: During a run, data are continuously stored on the operator’s computer and on the computer embedded in the instrument. If the communication between the instrument and the operator’s computer is interrupted during the run, data from the run will be automatically restored once communication has been re-established.
3. The temperature of the heating block and chamber rises to preset levels.
4. The mixer speed adjusts to the preset level.
5. The pressure in the pneumatic dispensing system rises to the preset level.

6. Once the temperatures and pressure reach the preset levels (this may take several minutes), the predispensation and dispensation of the reagents begins.
7. Enzyme and substrate mixtures are predispensed into the rectangular sink-well at the right-hand edge of the PyroMark Q96 Plate Low to ensure that the dispensation capillaries are flushed and filled with solution.
8. The reagent cartridge is positioned over each well in turn and the enzyme mixture is dispensed. The process is then repeated for the substrate mixture.

   Note: Dispensation of all reagents into the PyroMark Q96 Plate Low is in a zigzag fashion. The mixing system (vibrating process chamber) is operational throughout the sequencing run.
9. The dispensation pressure is increased.
10. The nucleotides are predispensed into the rectangular sink-well at the right-hand edge of the PyroMark Q96 Plate Low.
11. The reagent cartridge is positioned over each well in turn and the programmed nucleotide is dispensed.
12. The CCD camera collects data simultaneously from all wells throughout the process. The CCD camera is cooled to approximately 0°C to optimize performance and reduce background noise.
13. In wells where there is a positive reaction with the added nucleotide, light is emitted, giving rise to a peak in the Pyrogram®.
14. A period of 65 seconds (cycle time) is allowed between the addition of each nucleotide to ensure that all enzymatic reactions are completed.
15. The programmed sequence of nucleotide addition continues until the end of the program has been reached.

3.4 External features of the PyroMark Q96 ID

The PyroMark Q96 ID.

1 Instrument lid

2 Indicator LED

3 Standby switch

Note: The PyroMark Q96 ID is delivered with a laptop to serve as the operator's computer (see Section 3.8).

3.5 Internal features of the PyroMark Q96 ID

Internal view of the PyroMark Q96 ID.

1 Dispensing unit

2 Process chamber

3.6 Process chamber

The process chamber includes a Peltier heating block that maintains the correct temperature of the PyroMark Q96 Plate Low and its contents. An integral mixer enables rapid mixing of sample and reagents in the PyroMark Q96 Plate Low.

3.7 Dispensing unit

Reagents are manually added to the reagent cartridge, which is then inserted into the dispensing unit on top of the process chamber lid.

During a run, the dispensing unit is positioned over each well in the PyroMark Q96 Plate Low and reagents are dispensed in a zigzag fashion, using a pneumatic system.

3.8 Controls and indicators

3.8.1 Front panel

The standby switch is located on the lower left-hand side of the front panel. Three indicator lights can be found in the middle of the front panel:

- Top light: Power (green). When illuminated, this indicates the instrument has been connected to the mains electricity supply and is receiving power
- Middle light: Busy (yellow). When this light is illuminated or flashing, a run is in progress and the lid should not be opened
- Bottom light: Information (yellow). If this light is illuminated during a run, the instrument has lost contact with the operator's computer

During the start-up sequence:

- All the lights illuminate
- The instrument software loads (takes 1–3 minutes) and the "Busy" light goes off and the "Information" light starts flashing. This indicates that the instrument is ready to receive instructions from the operator's computer
- When an application is selected and "Run" is clicked in the "Setup Run" window, the flashing "Information" light will turn off and the "Busy" light will illuminate

3.8.2 Rear panel

The following connectors are located on the rear panel:

- Mains power input connector: Should be connected to a grounded mains power outlet
- Keyboard: Keyboard interface for service purposes. This should only be used by a certified QIAGEN Field Service Specialist
- Mouse: Mouse interface for service purposes. This should only be used by a certified QIAGEN Field Service Specialist
- Net: Ethernet network connector

- Monitor: Monitor interface for service purposes. This should only be used by a certified QIAGEN Field Service Specialist

3.9 Computer and software

The PyroMark Q96 ID is shipped with a laptop that serves as the operator's computer.

The computer used for data analysis should have the following as minimum specifications:

- Microsoft® Windows® XP 32 bit (English version) or Microsoft Windows 7 32 bit (English version) Operating System
- Intel® Core™ Duo processor (2 GHz) or higher
- 200 MB free hard drive capacity
- 1 GB RAM
- Ethernet ports (or external USB Ethernet adaptor)
- Monitor with 1280 x 800 pixels, True Color (32 bit)
- Graphics card supporting the resolution of the monitor
- Pointer device (mouse or similar)

4 Installation Procedures

4.1 Instrument delivery and installation

The unpacking and installation of the PyroMark Q96 ID is carried out by a certified QIAGEN Field Service Specialist. A member of your group who is familiar with laboratory and computer equipment should be present during the installation.

The following items are delivered:

- PyroMark Q96 ID instrument
- Operator's computer
- PyroMark Q96 Vacuum Workstation (must be ordered separately)
- *PyroMark Q96 ID User Manual* and other technical documentation in PDF format on accompanying CD-ROM
- PyroMark Q96 Software on accompanying CD-ROM

4.2 Requirements

Site

The PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation must be located out of direct sunlight, away from heat sources, and away from sources of vibration and electrical interference. Refer to Appendix A for the operating conditions (temperature and humidity). The site of installation should be free of excessive drafts, excessive moisture, excessive dust, and not subject to large temperature fluctuations.

Refer to Appendix A for the weight and dimensions of the PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation.

Ensure that the workbench is level, dry, clean, vibration-proof, and has additional space for accessories. Approximately 80 cm (31.5 in.) clearance above the workbench is required to accommodate the PyroMark Q96 ID with the lid open. Allow at least 10 cm (4 in.) of free space behind the instrument for cabling.

The PyroMark Q96 ID must be placed within approximately 1.5 m (59 in.) of a properly grounded (earthed) AC power outlet. The power line to the instrument should be voltage regulated and surge protected.

Note: It is recommend to plug the instrument directly into its own wall socket and not to share the socket with other lab equipment.

CAUTION	**Risk of overheating** [C1] To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the PyroMark Q96 ID. Slits and openings that ensure the ventilation of the PyroMark Q96 ID must not be covered.

Power requirements

The PyroMark Q96 ID operates at:

- 100–240 V AC, 50/60 Hz, 1.1–0.45 A.

Depending on the unit purchased, the PyroMark Q96 Vacuum Workstation operates at:

- 100 V AC, 50/60 Hz, 1.7/1.4 A
- 115 V AC, 60 Hz, 1.5 A
- 230 V AC, 50 Hz, 0.6 A

Ensure that the voltage rating of the PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation is compatible with the AC voltage available at the installation site. Mains supply voltage fluctuations are not to exceed 10% of nominal supply voltages.

Grounding requirements

To protect operating personnel, the PyroMark Q96 ID must be correctly grounded (earthed). The instrument is equipped with a 3-conductor AC power cord. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.

4.3 Moving the PyroMark Q96 ID

WARNING	**Risk of personal injury and material damage** [W2] The PyroMark Q96 ID is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

If you need to transport the PyroMark Q96 ID, package the instrument as follows:

1. When the instrument is not processing, shut down PyroMark Q96 Software and exit from Microsoft Windows.
2. Switch off the operator's computer and PyroMark Q96 ID and disconnect the mains power cable from the instrument.
3. To move the instrument to a new location within the same laboratory, go to step 4. To move the instrument to another site, go to step 7.
4. Place the instrument onto a trolley and move to the new location.

   Note: The instrument weighs 52 kg. Two persons are required.
5. Position the PyroMark Q96 ID on the bench. See Section 4.2 for site requirements.
6. Follow the reinstallation procedures in Section 4.4 for stand-alone installation or Section 4.5 for network installation.
7. To move the instrument to another site: First, mount the transport protection block under the instrument lid.
8. Pack the PyroMark Q96 ID and other components using the original packaging supplied with the instrument, if available.

   Note: The total weight of the package including the instrument is 80 kg. The instrument alone weighs 52 kg. Use suitable lifting equipment when moving the package.

9. At the new site, unpack the instrument and remove the transport protection block from under the instrument lid.
10. Follow the reinstallation procedures in Section 4.4 for stand-alone installation or Section 4.5 for network installation.

4.4 Reinstalling the PyroMark Q96 ID: Stand alone

When delivered, the PyroMark Q96 ID is configured as a peer-to-peer network. The operator's computer acts as the host and PyroMark Q96 ID as the guest.

Install a stand-alone system as follows:

1. Identify the cable marked "Cross-connected TP" provided with the PyroMark Q96 ID. Connect one end of this cable to the network connector on PyroMark Q96 ID ("net").

   Note: The "keyboard", "mouse", and "monitor" connectors are for the use of QIAGEN Field Service Specialists only.
2. Connect the other end of the Ethernet cable to the network card connector on the operator's computer.
3. Connect mains power as per Section 4.6.

4.5 Reinstalling the PyroMark Q96 ID: Network installation

The operator's computer is configured with a built-in Ethernet port to connect to the PyroMark Q96 ID and an external USB adaptor to connect to a Local Area Network (LAN).

The main advantages of connecting the PyroMark Q96 ID to a LAN are:

- Easier access to run data files
- Assays can be set up and analyzed on office computers
- Data such as SNP entries can be shared between different users
- Access to LAN printing resources

Install a LAN system as follows:

1. Connect one end of the cross-connected Ethernet network cable provided to the "NET" connector on the rear panel of the PyroMark Q96 ID.
2. Connect the other end of the network cable to the built-in LAN outlet on the operator's computer.
3. Connect the second Ethernet network cable between the Ethernet USB adaptor of operator's computer and the nearest LAN connection point.
4. Connect mains power as per Section 4.6.
5. Start up the operator's computer and assign a unique IP address to the second network adapter for communication with the LAN.

4.6 Connecting mains power

1. Ensure the standby switch on the front of the PyroMark Q96 ID is in position ⏻ (press the lower part of the switch).
2. Connect the mains cable to the inlet on the rear of the PyroMark Q96 ID. Connect the mains cable to a grounded mains power outlet. The PyroMark Q96 ID will automatically adjust to the correct voltage.

4.7 Setting up network properties

4.7.1 Used IP addresses for instrument and computer

The instrument IP address and the host IP address (operator's computer) are fixed and do not need to be changed if the PyroMark Q96 ID is connected to a LAN.

- Operator's computer IP address: 192.168.255.200
- PyroMark Q96 ID IP address: 192.168.255.201

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5 Operating Procedures

This section describes how to operate the PyroMark Q96 ID.

Before proceeding, it is recommended that you familiarize yourself with the features of the PyroMark Q96 ID by referring to Sections 3.4 and 3.5.

5.1 Required items

The following items are needed to perform a run:

- Operator's computer loaded with PyroMark Q96 Software
- PyroMark Q96 Vacuum Workstation
- Sample preparation buffers
- PyroMark Gold Q96 Reagents
- PyroMark Q96 ID Cartridge
- PyroMark Q96 Plate Low
- High-purity water (Milli-Q® 18.2 MΩ x cm or equivalent)
- Powder-free gloves
- Lint-free tissues

5.2 Starting the system

The PyroMark Q96 ID requires a warm-up time of 90 minutes for the CCD camera output to stabilize. To save time, switch on the instrument before starting to prepare samples.

5.2.1 Starting the PyroMark Q96 ID

Before starting the PyroMark Q96 ID, ensure that the mains power cable is correctly inserted into the mains power connector on the rear panel and connected to a grounded mains power socket.

Turn the power switch to **I** (On). All indicator lights turn on when power is connected. After 1–3 minutes, the "Busy" light will turn off and the "Information" light will start flashing. The instrument is now ready for a run.

When a run is started, the flashing "Information" light will turn off and the "Busy" light will illuminate. Keep the lid closed during a run.

If the "Information" light illuminates during a run, the operator's computer has lost contact with the instrument.

5.2.2 Starting the PyroMark Q96 Software

1. In the Windows "Start" menu, select "(All) programs/PyroMark/PyroMark Q96".
2. The *PyroMark Q96 Software User Guide* can be accessed at any time by pressing the "F1" key when in the software.

5.3 Preparing a run

5.3.1 Setting up an assay

1. In the shortcut browser, right-click the folder you want to place the assay in, and select "New Assay" followed by the desired assay type (AQ, CpG, SNP, or SQA) from the context menu.
   Note: To add a shortcut to a folder or drive, click "Add Folder Shortcut".
2. Click in the toolbar to enter a run name and save the file in the desired folder.

   Note: You cannot use the same name as another file saved in the same folder.
3. If creating an AQ, CpG, or SNP assay, type or paste the sequence to analyze into the "Sequence to Analyze" text box and then click the "Generate Dispensation Order" button. If creating a CpG assay, enter the sequence after the bisulfite treatment. If entering an SQA assay, enter the dispensation order.
4. Click in the toolbar.

   Note: Before running your samples, validate your assay using a reference DNA sample.

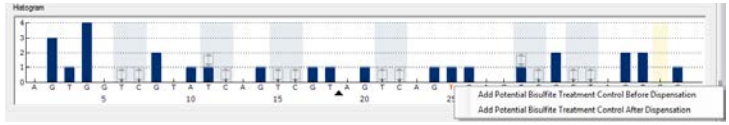
   Note: When using QIAGEN kits, use the settings stated in the kit handbooks.

Optional

If desired, enter a note about the assay and set up the variable positions in the "Variable Positions" tab.

If creating a CpG assay, it is recommended that bisulfite treatment controls are added. In the sequence before bisulfite treatment, check if the suggested bisulfite controls are **C**s converted to **T**s (read as **G**s and **A**s in a reverse assay) and are suitable for controls or not.

To add a control, left-click a bold, orange **T** or **A** in the histogram, preferable at the beginning of the sequence.


Histogram
Add Potential Bisulfite Treatment Control Before Dispensation
Add Potential Bisulfite Treatment Control After Dispensation

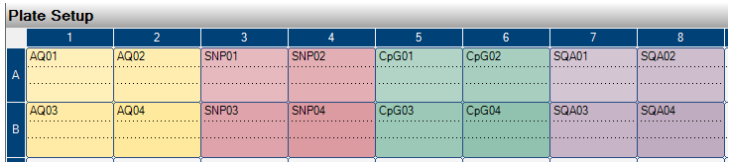
If applicable, during the assay validation, edit the analysis parameters.

5.3.2 Setting up a run

1. In the shortcut browser, right-click the folder you want to place the run file in, and select "New Run" from the context menu.

   Note: To add a shortcut to a folder in the shortcut browser, click "Add Folder Shortcut".

2. Click in the toolbar to enter a run name and save the file in the desired folder.
3. In the "Instrument Parameters" drop-down list, select the instrument parameters file that corresponds to the PyroMark Q96 Cartridge to be used.
4. **Optional**: Enter the "Reagent ID", "Plate ID", plate "Bar code", or a "Run note".
5. Add an assay to each well used, e.g., drag an assay from the shortcut browser to a well or a selection of wells. A well is colored according to the assay loaded into the well. Alternatively, right-click a well or selection of wells and select "Load Assay".



6. Click in the toolbar to save the run setup.
7. Print a list of required volumes of reagents and the plate setup by selecting "Pre Run Information" from the "Tools" menu, and when the report opens, clicking .

Further information

There are several ways to set up a plate. For example, it is possible to import and paste a sample layout defined in a text file, and drag-copy and increment a sample ID (if the last part of the entered sample ID is a number). For more information, see the *PyroMark Q96 Software User Guide* (press the "F1" key when in the PyroMark Q96 Software).

Note: To base your run on a previous run, right-click the processed run file in the shortcut browser and select "Copy and Rerun" from the context menu. Only the run setup, not the run and analysis data will be copied.

5.3.3 Managing instrument parameters

Instrument parameters should be selected according to the reagent cartridge that will be used for the run. The code number printed on the PyroMark Q96 Cartridge corresponds to specific instrument parameter settings provided at www.qiagen.com/Products/PyroMarkQ96ID.aspx.

Note: We recommend that only instrument parameters supplied by QIAGEN are used.

Important: In order to access instrument parameter settings online, you must register your instrument with a valid serial number (see back of instrument) at www.qiagen.com.

Managing instrument parameters in PyroMark Q96 Software:

1. Select "Instrument Parameters" from the "Tools" menu to open the instrument parameters dialog box.

2. Right-click an existing instrument parameter file listed in the upper pane and select “Duplicate” from the context menu.
3. Enter a new file name (e.g., “Instrument parameters Code 0005”).
4. Go to the Web page cited above and open the file “Managing instrument parameters for the PyroMark Q96 ID”, found under the User Support tab. In the provided table, find the dispensing pressures and pulse time settings that correspond to the Code number printed on the PyroMark Q96 Cartridge to be used.
5. Enter the dispensing pressures and pulse time into the corresponding fields of the “Instrument Parameters” dialog box.
6. Save the new instrument parameter file.
7. When creating a new Run Setup, select this new instrument parameter file from the list of instrument parameter files.

Instrument parameters

In the “Instrument Parameters” dialog box, the following parameters are available.

Reagent pressure	Pressure (millibar) for dispensation of the enzyme mix and substrate mix.
Enzyme pulse time	Dispensation time (milliseconds) for the enzyme mix.
Substrate pulse time	Dispensation time (milliseconds) for the substrate mix.
Nucleotide pressure	Pressure (millibar) from the dispensation of nucleotides.
Nucleotide pulse time	Dispensation time (milliseconds) for nucleotides.
Note	Note about the instrument method (optional).

5.4 Sample preparation

Equipment and reagents to be supplied by the user

- PyroMark Q96 Vacuum Workstation
- PyroMark Q96 Plate Low (cat. no. 979002)
- PyroMark 96 Sample Prep Thermoplate Low (supplied with the PyroMark Q96 ID)
- Mixer for immobilization to beads
- Heating block capable of attaining 80°C
- 96-well PCR plate and adhesive foil or strip caps
- Biotinylated PCR template
- Streptavidin Sepharose® HP (GE Healthcare, cat. no. 17-5113-01)
- Sequencing primer
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Ethanol (70%)

- PyroMark Binding Buffer (cat. no. 979006)
- PyroMark Denaturation Solution (cat. no. 979007)
- PyroMark Wash Buffer concentrate (cat. no. 979008)
- PyroMark Annealing Buffer (cat. no. 979009)

5.4.1 Assembly of the PyroMark Q96 Vacuum Workstation

Assemble the PyroMark Q96 Vacuum Workstation as follows:

1. Place the PyroMark Q96 Vacuum Workstation on a flat surface.
2. Connect vacuum tubing to the straight blue connector on the cap of the liquid waste container. Attach the other end of this tubing to the vacuum pump.
3. Connect a second piece of vacuum tubing to the angled blue connector on the cap of the liquid waste container. Attach the other end to the connector on the back of the PyroMark Q96 Vacuum Workstation.
4. With a third piece of tubing, attach the Vacuum Prep Tool to the vacuum tubing connector on the top of the PyroMark Q96 Vacuum Workstation (see image below).
5. Test the PyroMark Q96 Vacuum Workstation by switching on the vacuum pump and opening the switch on the front of the workstation. If not vacuum is created, turn off the vacuum pump and open the cap of the liquid waste container to release any built-up air pressure.

   Note: The liquid waste container must be able to withstand absolute vacuum.

5.4.2 DNA amplification

Amplify the DNA to be analyzed by PCR using one of the primers biotinylated. See Appendix B for details on assay design and validation.

5.4.3 Immobilization of the PCR product to beads

Biotinylated PCR products are immobilized on streptavidin-coated Sepharose beads (Streptavidin Sepharose High Performance, GE Healthcare). For each sample the final immobilization volume is 80 µl. The reaction setup is shown in the table below.

Component	Volume per sample	
	For lot number 10057037 or higher	For lot numbers lower than 10057037
Biotinylated PCR product	5–20 µl	5–20 µl
Streptavidin Sepharose HP*	1.5 µl	3 µl
PyroMark Binding Buffer	40 µl	40 µl
High-purity water†	Variable†	Variable†
Total volume	**80 µl**	**80 µl**

* Check the lot number of the Streptavidin Sepharose HP. For lot number 10057037 or higher use 1.5 µl. For lot numbers lower than 10057037, use 3 µl.

† The volume of water depends on the amount of PCR product used.

Allow all reagents and solutions to equilibrate to room temperature (15–25°C) before starting. All steps are performed at room temperature, unless stated.

1. Gently shake the bottle with streptavidin-coated Sepharose beads from side to side until a homogenous solution is obtained.
2. Prepare a mixture of Streptavidin Sepharose beads, Binding Buffer, and high-purity water sufficient for all samples to be analyzed.
3. Add the mixture prepared in step 2 to the wells of a 96-well PCR plate according to the plate setup (see Section 5.3.1).
4. Add 5–20 µl of a well-optimized, biotinylated PCR product to each well used.
   Note: The total volume per well should be 80 µl.

Note: If performing multiplex analysis of pooled PCR products, use 5–10 µl of each product and reduce water volume to maintain the total volume. The total volume should be 80 µl after addition of the master mix and PCR product.

5. Seal the PCR plate using strip caps. Ensure that no leakage is possible between the wells.
6. Agitate the PCR plate constantly for at least 5–10 min using a mixer (1400 rpm).
   Note: Sepharose beads sediment quickly and capturing of beads must take place immediately once the agitation is complete. If more than 1 minute has elapsed, agitate the plate again before capturing the beads.
   Note: During immobilization, prepare the Vacuum Workstation for the sample preparation.
7. Proceed with strand separation, see next section.

5.4.4 Strand separation

WARNING	**Hazardous chemicals** [W4] The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Procedure:

1. Ensure that the PyroMark Q96 Vacuum Workstation has been assembled correctly and securely (see "Assembly of PyroMark Q96 Vacuum Workstation", page 5-7). The mains plug should be easily accessible in case the vacuum pump needs to be disconnected quickly from the mains power.

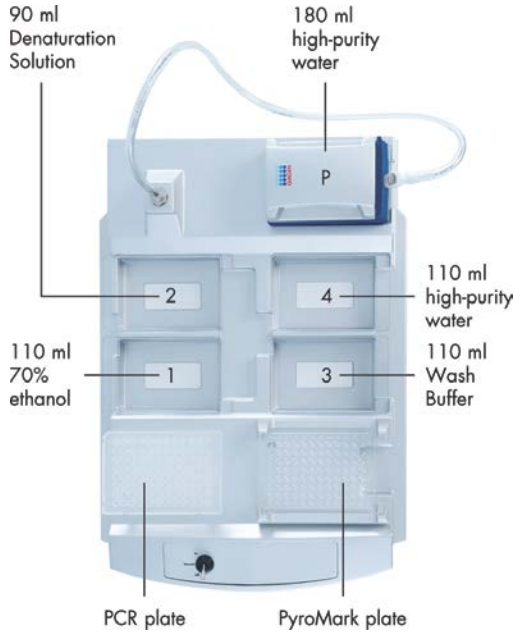
   Note: Perform the function test to ensure that the filter probes are working properly (see Section 7.8.1). All probes should be replaced after preparation of approximately 100 plates, or when needed.

   Note: Check and empty the waste container, if necessary (see Section 5.7.5).

2. Fill five separate troughs (supplied with the PyroMark Q96 Vacuum Workstation) as follows:
   - Approximately 110 ml ethanol (70%) (1)
   - Approximately 90 ml Denaturation Solution (2)
   - Approximately 110 ml Wash Buffer (3)
   - Approximately 110 ml high-purity water (4)
   - Approximately 180 ml high-purity water (Parking Position, "P").

   A suggested setup is shown on the next page. Refill the troughs to these levels whenever necessary.

   The total volume that can be added to each trough is 180 ml. There is a mark on each trough indicating 90 ml.



3. Switch on the vacuum pump.
4. Apply vacuum to the tool by opening the vacuum switch.
5. Wash the filter probes by lowering the probes into high-purity water (Parking Position). Flush the probes with 180 ml high-purity water.
   Ensure that the water is being transferred to the waste container. If it is not, ensure the tubing is connected correctly and is not broken. Broken tubing should be replaced, see Section 7.9.
   Ensure that the tubing is connected properly and there is no leakage.
6. Refill Parking Position with 180 ml high-purity water.
7. Close the vacuum switch on the worktable (Off) and place the tool in the Parking Position.
8. Add 40 µl of 0.4 µM sequencing primer in Annealing Buffer to each well of the PyroMark Q96 Plate Low that is to be used.

9. After shaking, place the PCR plate and PyroMark Q96 Plate Low on the worktable. Ensure that both plates are in the same orientation as when samples were loaded.
   Note: Sepharose beads sediment quickly. If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.
10. Apply vacuum to the tool by opening the vacuum switch.
11. Carefully lower the filter probes into the PCR plate to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.
12. Ensure that all liquid is aspirated from the wells and that all beads have been captured onto the filter probe tips.
    Note: If the wells still contain liquid or white beads remain, the filter probes may need replacing, see Section 7.8.2.
13. Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.
14. Transfer the tool to the trough containing Denaturation Solution (trough 2). Flush the filter probes for 5 s.
15. Transfer the tool to the trough containing Wash Buffer (trough 3). Flush the filter probes for 10 s.
16. Raise the tool to beyond 90° vertical for 5 s, to drain liquid from the filter probes (see image).

17. While holding the tool over the PyroMark Q96 Plate Low, close the vacuum switch on the worktable (Off).
18. Release the beads in the plate containing 0.4 µM sequencing primer in 40 µl Annealing Buffer by shaking the tool gently from side to side in the plate. Allow the filter probes to rest on the bottom of the wells.
19. Transfer the tool to the trough containing high-purity water (trough 4) and agitate the tool for 10 s.
20. Wash the filter probes by lowering the probes into high-purity water (Parking Position) and applying vacuum. Flush the probes with 180 ml high-purity water.
21. Raise the tool to beyond 90° vertical for 5 s, to drain liquid from the filter probes.
22. Close the vacuum switch on the worktable (Off) and place the tool in the Parking Position.
23. If more than one plate is prepared at once, refill the troughs (step 2) and repeat the procedure from step 8.
24. Turn off the vacuum pump.

Note: The filter probes should be left to air dry when not in use, i.e., empty the trough and ensure there is no water in the Parking Position when the tool is left there.

5.4.5 Annealing of sequencing primer to samples

WARNING	**Hot surface** [W8] The thermoplate and the heating block (for annealing) can reach temperatures of up to 80°C (176°F). Avoid touching them when they are hot.

1. Place the PyroMark Q96 Plate Low in a prewarmed PyroMark Q96 Sample Prep Thermoplate Low, and heat the samples on a heating block at 80°C for 2 min.
2. Remove the plate from the thermoplate and allow the samples to cool to room temperature (15–25°C) for at least 5 min. The plate can now be processed in the PyroMark Q96 ID.

5.4.6 Reagent preparation

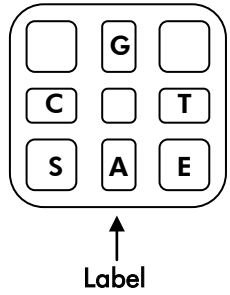
1. Prepare reagents according to instructions included with the PyroMark Gold Q96 Reagents.
2. Open a reagent cartridge pack and take out a new cartridge. The cartridge has to be handled with care in order to maintain accuracy.
3. Remove the plastic needle cover from the underside of the reagent cartridge. Save the cover for use when disposing of the reagent cartridge. Do not use the plastic needle cover between runs, since applying and removing the cover several times may damage the needles.

   Note: If re-using a reagent cartridge, ensure that it is cleaned appropriately, see Section 7.6. If handled correctly, a reagent cartridge can be used for up to 20 runs.

   Note: It is important that the reagents are pipetted into the correct reagent cartridge compartments.
4. Place the reagent cartridge with the label facing towards you. Pipet the recommended volume of substrate solution into the **S** compartment of the reagent cartridge.

5. Pipet the recommended volume of enzyme solution into the **E** compartment
6. Pipet the recommended volume of each dNTP solution into the **A**, **C**, **G**, and **T** compartments.

   Note: do not use filtered tips to pipet nucleotides.



PyroMark Q96 Cartridge (from above) with indicated compartments for enzymes, substrate, and nucleotides.

5.5 Run

5.5.1 Loading the PyroMark Q96 Plate Low

1. Open the instrument lid.

   Note: The dispensing unit and process chamber will move if a run is accidentally started.
2. Open the process chamber lid.
3. Open the plate-holding frame.
4. Place the PyroMark Q96 Plate Low on the heating block.
5. Close the plate-holding frame and the process chamber.

5.5.2 Loading the reagent cartridge

1. Open the dispensing unit cover: Release the latch and then open the cover.
2. Insert the filled reagent cartridge into position. It will only fit in one orientation; the label should be facing the user.
3. Close the dispensing unit cover. Ensure the latch snaps into its locked position.
4. Close the instrument lid.

5.5.3 Starting the run

Start the run by clicking . The "Run" view becomes active. The run can be monitored, paused, or stopped in this view.

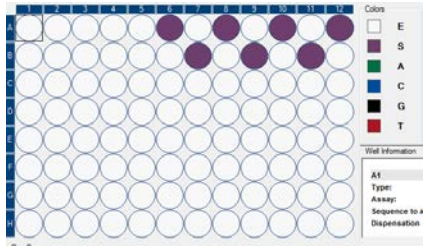
Note: The "Start" icon is enabled only if an instrument connection has been configured, instrument parameters have been defined for the run file, and at least one well has an assay.

5.5.4 Monitoring the run

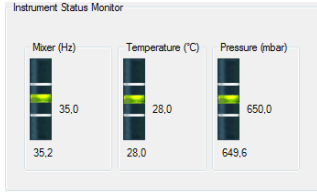
The "Run" view displays the following information regarding the progress of a run:

- Last nucleotide or reagent dispensation to each well
- Well-specific information
- Instrument-specific information in the Instrument Status Monitor

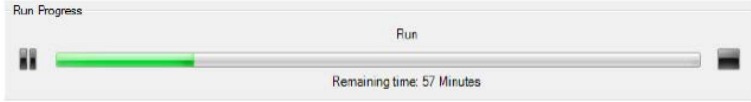
A diagram of a 96-well plate displays all wells active in the run. During the course of the run, each well is colored to reflect the last reagent (Enzyme Mix or Substrate Mix) or nucleotide (A, C, G, or T) dispensed to the well. A legend indicates which reagent corresponds to each color.



Mixer frequency, temperature of the process chamber, and pressure in the dispensing unit are displayed in real-time in the "Instrument Status Monitor".



A run progress bar displays the current state of a run (e.g., preparing for run, adding reagents, run, end of run, connection lost) and the time remaining to complete the run.



Warnings during a run

Errors may occur during a run, which triggers warnings that will appear in the "Warnings" pane. The warning is also documented in the Run Log of the "Run Information". If an error occurs during a run, a dialog box that appears at the end of the run gives the user the option to review the Run Information.

Restoring a run

If the connection to the instrument is lost during a run, a message box will appear to inform the user. Upon clicking "OK", the "Run" view will close, but the instrument will continue to process the run. The data collected will be stored on the instrument, and the run file can be restored. See the *PyroMark Q96 Software User Guide* for more information.

5.6 Post-run procedures

5.6.1 Analyzing the run

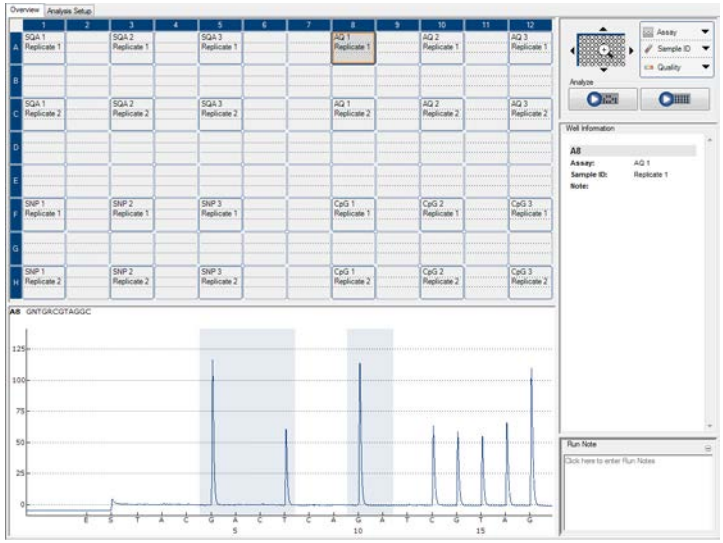
Detailed instructions for analyzing the run are available in the *PyroMark Q96 Software User Guide* (press the "F1" key when in PyroMark Q96 Software).

Analysis modes

PyroMark Q96 Software has four analysis modes: AQ, SNP, CpG, and SQA. To toggle between the modes, select "AQ", "SNP", "CpG", or "SQA" in the toolbar.

Note: How the analysis is performed can be modified using the "Analysis Setup" tab.

Overview tab



1. In the “Overview” tab, click the “Zoom Tool” to zoom into a quadrant of the 96-well plate. Use the arrow around the zoom tool or click the desired quadrant of the plate to change which 24 wells are displayed.
2. Click the “Analyze Selected Wells” button to analyze the selected wells with a valid analysis setup for the current analysis mode, or click the “Analyze all Wells” icon to analyze all wells.

   Analyze all wells

   Analyze the selected wells only

5.6.2 Viewing the analysis results

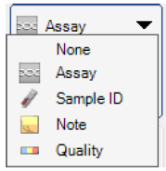
By selecting an analyzed (light blue) well in the “Overview” tab, the corresponding Pyrogram and theoretical histogram (if an AQ, SNP, or CpG assay) or compensated Pyrogram (if an SQA assay) are displayed in the “Pyrogram” pane. The well information (including analysis warnings) is listed in the “Well Information” area. If several wells are selected in the plate overview, information for the well with the orange selection frame is shown.

Pyrograms from 2 or more wells can be viewed simultaneously by opening one or more additional Pyrograms of selected wells in the lower area of the "Pyrogram" pane.

Get an overview of the results

The following well information can be viewed in the plate overview in the "Overview" tab:

AQ and SNP assays



Select to show the assay name

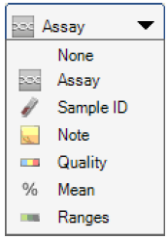
Select to show the sample ID.

Select to show the well note.

Select to show the quality bar. The quality bar shows the quality assessment of all variable positions in the well or of all the bases in the base-called sequence.

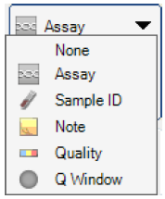
CpG assays

Select to show the mean methylation percentage of all CpG sites in the well.



Select to show the methylation bar. The methylation bar shows the methylation level for each CpG site in the well.

SQA assays



Select to show the quality assessment at the end of the quality control window. The default number of bases included is 20.

Quality assessments

The plate overview in the "Overview" tab summarizes quality assessments. Select the "Quality" option in the upper right corner to display the quality assessment of all variable positions in a selected well or of all bases in the base-called sequence. SQA assays also include quality assessment at the end of the quality control window. Quality levels are indicated with different colors:

- Blue: Passed
- Yellow: Check
- Red: Failed
- White: Not analyzed. Either analysis is not supported by the software (e.g., analyzing single nucleotide polymorphisms in CpG mode) or the variable position has been deselected by the user (AQ, SNP, and CpG assays only).

AQ, SNP, and CpG analysis results

Allele frequencies (AQ analysis), genotypes (SNP analysis), and methylation percentages (CpG analysis) are displayed at

the top of the Pyrogram. The quality assessment is indicated by the background color of the result. Variable regions are highlighted with a blue-gray background color.

In CpG analysis, bisulfite treatment controls are highlighted in the Pyrogram with a light yellow background color. Select the "Ranges" option in the upper right corner of the "Overview" tab to show methylation bars in the plate overview. These bars indicate the methylation level for each CpG site in a selected well, as compared to the expected level. Expected ranged can be set under the "Analysis Setup" tab.

- Light green: Below the expected range
- Green: Within the expected range
- Dark green: Above the expected range

SQA analysis results

The base-called sequence is displayed in the "Overview" tab above the Pyrogram of the selected well. The bases in the base-called sequence and the peaks in the compensated Pyrogram in the lower window of the "Pyrogram" pane are colored according to their quality assessments.

5.6.3 Analysis reports

To generate a report, select the desired report from the "Reports" menu. For more information about reports, see the "View, Print, and Save Analysis Reports" section of the *PyroMark Q96 Software User Guide* (press the "F1" key when in the PyroMark Q96 Software).

Note: To view reports generated in PDF format, a PDF reader must be installed on the computer. Adobe® Reader® can be downloaded at www.adobe.com.

5.7 Finishing work

5.7.1 After a run is finished

Remove and dispose of the reagent cartridge

1. Open the instrument lid.
2. Open the dispensing unit cover.
3. Remove the reagent cartridge.
4. Close the dispensing unit cover. Ensure that the latch snaps into its locked position.

   Note: If the reagent cartridge is to be reused, clean it directly after use, as described in Section 7.6.

WARNING	**Sharp needles** [W7] Do not touch the sharp needles at the bottom of the reagent cartridge. Handle the needles with care. Small particles and fibers may obstruct the needles.

Note: To avoid injury, replace the plastic needle cover on the reagent cartridge prior to disposal.

Note: Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

Remove and dispose of the PyroMark Q96 Plate Low

1. Open the process chamber lid.
2. Open the plate-holding frame and remove the PyroMark Q96 Plate Low from the heating block.
3. Close the plate-holding frame and the process chamber lid.

   Note: Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

5.7.2 Leaving the instrument on

To ensure stable operating conditions, the PyroMark Q96 ID should remain on between runs.

Shut down the instrument only if it will not be used again within two weeks. See Section 7.1 for shutdown instructions.

5.7.3 Exiting PyroMark Q96 Software

Exit the PyroMark Q96 Software by choosing “Exit” from the “File” drop-down menu.

5.7.4 Cleaning the instrument

Remove any spillages around the dispensing unit and process chamber with a clean, lint-free cloth. See Sections 7.2 through 7.6 for detailed cleaning instructions.

5.7.5 Emptying the waste container and troughs

WARNING	**Hazardous chemicals** [W4] The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

The following item is required:

- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent).

Procedure:

1. Ensure that no vacuum is applied to the Vacuum Prep Tool, i.e., the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Discard any solutions left in the troughs.
3. Rinse the troughs with high-purity water, or replace them, if necessary.
4. Empty the waste container.
   Note: The cap can be removed without disconnecting the tubing.
5. If the vacuum workstation must be cleaned (e.g., dust or spillage), follow the instructions in Section 7.7.

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6 PyroMark Q96 Software

The operator's computer is configured for communication with the PyroMark Q96 ID Instrument.

6.1 System requirements

6.1.1 Operator's and office computers

See Section 3.9 for minimum specifications.

6.1.2 Printer

All printers supported by the operator's computer are suitable. QIAGEN recommends the use of a laser printer.

6.1.3 Data storage device

A removable hard drive or similar should be used for backing up the result files. This is required if the computer used to analyze the data is not connected to a LAN.

6.2 Installing PyroMark Q96 Software

Note: If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your system administrator.

1. Close any programs running on the computer.
2. Insert the PyroMark Q96 ID Application Software 2.5 CD into the CD drive.
3. In the CD menu, click "Install PyroMark Q96 Software".

   If the CD menu does not appear automatically:
   - Select "(My) Computer" in the Windows "Start" menu.
   - Right-click the CD drive with the software CD and select "Explore".
   - Double-click the file autorun.exe.
4. Follow the instructions that appear in the "Setup Wizard".

Note: If .NET Framework 3.5 has to be installed (installation is prompted by the "Setup Wizard"), the installation must be restarted after the .NET Framework installation has been completed, i.e., open the CD menu (see step 3) and click "Install PyroMark Q96 Software".

5. When the software has been successfully installed, click "Exit Setup" in the CD menu.
6. Please use Windows Update (www.update.microsoft.com) to check for any critical updates to the .NET Framework 3.5.

6.3 Uninstalling PyroMark Q96 Software

1. Select "Control Panel" in the Windows "Start" menu.
2. If using Microsoft Windows XP, click "Add or Remove Programs"; if using Microsoft Windows 7, click "Programs" and then "Programs and Features".
3. In the list of programs, select "PyroMark Q96".
4. Click "Remove" (XP) or "Uninstall" (7).

6.4 Upgrading from PyroMark Q96 ID Software

Previous versions of PyroMark Q96 ID Software stored Pyrosequencing runs in an Oracle database. Users upgrading to the new PyroMark Q96 Software must first use the PyroMark Data Converter Tool to convert this run database into a format that is compatible with the new software.

Note: This procedure should only be followed by users upgrading from the old PyroMark Q96 ID Software.

Important: The PyroMark Data Converter Tool is only compatible with Microsoft Windows XP. Users also planning to upgrade to Windows 7 must convert their data into the new format before upgrading their operating system. Runs which cannot be converted (refer to 6.4.2) cannot be accessed with the new PyroMark Q96 Software.

6.4.1 Installing the PyroMark Data Converter Tool

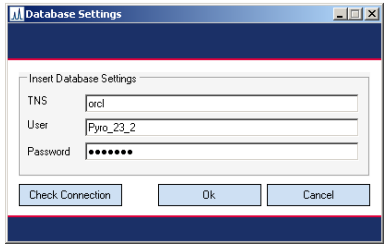
1. The PyroMark Data Converter can be downloaded at www.qiagen.com/goto/PyroMarkDataConverter at no cost. In order to access this page and download the software, you must register your instrument with a valid serial number (see back of instrument) at www.qiagen.com/PyroMarkRegistration.
2. Install the PyroMark Data Converter software on the instrument operating computer containing the database of runs to be converted. This computer must be running the Microsoft Windows XP operating system.

6.4.2 Using the PyroMark Data Converter Tool

The PyroMark Data Converter tool converts Pyrosequencing runs set up and completed with the old PyroMark Q96 ID Software into a file format that is compatible with the new PyroMark Q96 Software. This tool copies the run data, converts the data to the new format, and exports the converted data to the location specified by the user. Only sequence analysis (SQA) and genotyping (SNP) runs which have been executed and completed will be converted. CpG assays generated with the PyroMark CpG Software do not need to be converted. These runs are in a format that is compatible with the new PyroMark Q96 Software. Runs containing multiplex assay data cannot be converted and are skipped during the conversion procedure.

1. Open the PyroMark Data Converter and open the Database Settings window by clicking “Connect” or selecting “Connect” from the “File” menu.

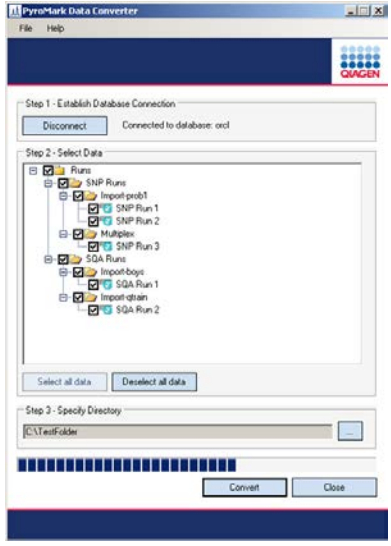
2. Enter the requested information — the database name (TNS), user name, and password — into the dialog box, and click the "OK" button to connect to the specified database.



Note: Click "Check Connection" to determine if a connection is possible with the information entered.

Note: The database connection can be disconnected by clicking "Disconnect".

3. Select Pyrosequencing data for conversion by checking the box next to individual runs or folders, or using the "Select all data" or "Deselect all data" buttons.



4. Click ..., and select or create a new directory in which to save the converted data. The path to the selected directory will be shown in the text field.
5. Click "Convert" to convert and export all selected data to the specified directory. The progress of the export will be displayed in the progress bar.

6.5 Data backup

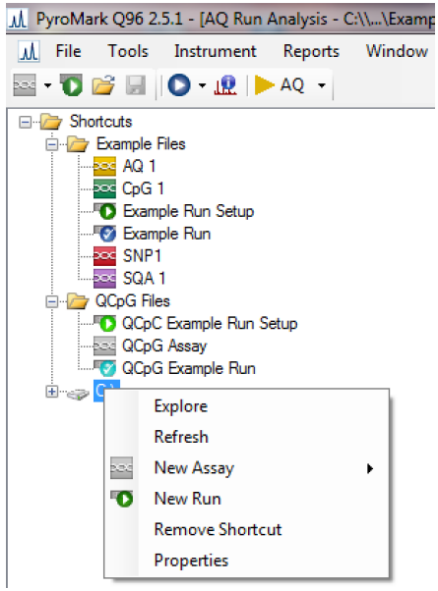
Good data management requires that data backups are made on a regular basis.

Note: QIAGEN is not responsible for the setup and administration of the user's backup routine.

6.6 Installation testing

Stand-alone systems and LAN systems should be tested after installation using the test method below. This test will check the main functions of the hardware and software. If the test functions correctly, the PyroMark Q96 ID can be used for running samples.

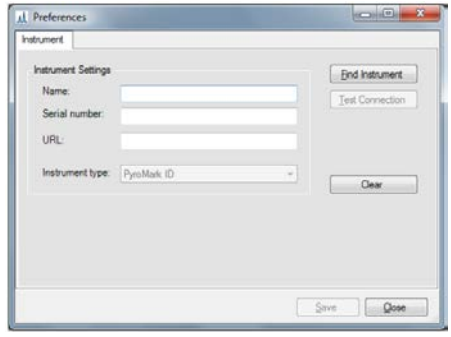
1. Start the operator's computer. Microsoft Windows starts and displays the welcome screen.
2. Press "Ctrl" + "Alt" + "Delete" and log on as the Administrator. No password is required. The Windows desktop will appear.
3. Start the PyroMark Q96 ID using the standby switch on the front panel. All lights on the front panel will illuminate. The instrument software is loaded onto the PyroMark Q96 ID. This takes 1–3 min. The yellow "Busy" light on the instrument will then go out and the yellow "Information" light will start to flash. The instrument is now ready to receive instructions from the operator's computer.
4. Start the PyroMark Q96 Software from the "Start" menu.
5. In the shortcut browser, right-click the folder in which to place the installation test run file, and select "New Run" from the context menu.



6. Click in the toolbar to enter a run name and save the file in the desired folder.
7. Add an assay to all wells, e.g., select all wells and drag an assay from the shortcut browser to the selection.

Note: If there are no existing assays in the shortcut browser, refer to section 5.3.1 to set up a new one.

8. In the "Instrument Parameters" drop-down list, select the instrument parameters file that corresponds to the PyroMark Q96 Cartridge to be used.
9. Click to save.
10. Open the instrument lid.
11. Open the process chamber lid.
12. Place an empty PyroMark Q96 Plate Low on the heating block.
13. Close the process chamber lid.
14. Open the dispensing unit cover and insert an empty reagent cartridge.
15. Close the dispensing unit cover and press the latch down into its locked position.
16. Close the instrument lid.
17. Check that the instrument is connected by selecting "Preferences" from the "Tools" menu.



Note: If the instrument name does not appear, click "Find Instrument" to have the software detect the connected PyroMark Q96 ID Instrument. Upon detecting the instrument, the software will enter the instrument serial number and URL. Assign a name to the instrument and click "Save".

18. Start the run by clicking , and check that the run starts. The flashing "Information" light will go out and the "Busy" light will illuminate.
19. The "Run" view becomes active, and the run progress bar displays the current state of the run.

    The chamber and heating block temperatures and pressure will stabilize to preset values. When these values are reached, dispensation of the enzyme (beige) and substrate (purple) begins.
20. When dispensation of enzyme and substrate is complete, the pressure increases to the nucleotide pressure value defined in the Instrument Parameters. The run progress bar displays "Run", and dispensation of the nucleotides begins.
21. Function test: Open the instrument lid.

    There will be an audible warning signal and the yellow "Information" light will illuminate.
22. Open the process chamber lid.

    The X-Y table should move at a rate of about 1 well every 0.6 s; the pneumatic dispensation system should deliver an air pulse every 0.6 s; the heating block should oscillate (vibrate).
23. Close the process chamber lid and the instrument lid. The "Information" light on the instrument will go out and the audible warning signal will cease.
24. Click on each well in "Well overview". The intensity level in the Pyrogram should be about 4000 for all wells.
25. Click "Stop" in the run progress bar. Click "Yes" in the dialog box that appears. The run will be stopped immediately and the data will be saved.

7 Maintenance

The following maintenance procedures must be carried out to ensure reliable operation of the PyroMark Q96 ID:

- Regular performance checks
- Cleaning of the instrument

Following these procedures ensures that the PyroMark Q96 ID is free of dust and liquid spills.

Before undertaking maintenance procedures, it is recommended that you familiarize yourself with the safety information in Section 1.

Important: Disconnect the instrument from mains power before cleaning.

Servicing

The PyroMark Q96 ID is supplied with a warranty that lasts for 1 year, beginning on the date of shipment. The warranty includes all repairs due to mechanical breakdown. Application development, software upgrades, accessories, and disposable items are not included in the warranty.

QIAGEN offers comprehensive Service Support Agreements, including Warranty Extensions, Full Cover Support Agreements, and instrument/application training, including on-site installation and annual preventative maintenance. Service Support Agreements maximize productivity and ensure high performance from your instrument. In addition, service histories are fully documented and all parts are certified and guaranteed.

Contact your local QIAGEN Field Service Specialist or your local distributor for more information about flexible Service Support Agreements from QIAGEN.

7.1 Shutdown procedure

Before carrying out maintenance, ensure the PyroMark Q96 ID is shutdown using the following procedure:

1. Choose “Instrument” from the main menu.
2. Choose “Shut Down Instrument”.
3. Click “Yes” to confirm shutdown of the instrument. Error messages will appear if the selected instrument is not connected or the selected instrument is busy.
4. All indicators on the front panel illuminate and the shutdown procedure begins. Shutdown is complete when the message “Now it is safe to turn off instrument” is displayed.
5. Turn off the standby switch on the front of PyroMark Q96 ID and check that the Power indicator lamp is turned off.

7.2 Routine maintenance of the PyroMark Q96 ID

The PyroMark Q96 ID should be checked and cleaned regularly to remove dust and spillages.

Important points before starting:

- Avoid harsh cleaners and chemicals, and getting moisture inside the instrument
- The cleaning liquid must be applied to the cloth only

The following items are required:

- Ethanol (70%)
- Clean, nonabrasive, lint-free cloths

Procedure:

1. Shut down the instrument, see Section 7.1.
2. Open the instrument lid.
3. Clean the area around the dispensing unit and process chamber using a lint-free cloth moistened with 70% ethanol.
4. Open the process chamber lid. Clean the area around the heating block.

7.3 Cleaning the lens array

The following items are required for cleaning the lens array beneath the heating block:

- Ethanol (70%)
- Clean, nonabrasive, lint-free cloths

Procedure:

1. Shut down the instrument, see Section 7.1.
2. Open the instrument lid.
3. Loosen the bayonet screw behind the heating block by turning it ¼ turn counter-clockwise until a click is heard.
4. Lift out the heating block. Avoid damaging the contact pins on the underside of the block.
5. Carefully clean the lens array with a cloth moistened with 70% ethanol.
6. Let the lens array dry before remounting the heating block.
7. Place the heating block on top of the lens array. Ensure that the rear part of the heating block is properly seated over the contact pins. Do not force the heating block into place.
8. Turn the bayonet screw ¼ turn clockwise.

7.4 Cleaning the heating block

The following items are required for cleaning the heating block:

- Ethanol (70%)
- Cotton swabs

Procedure:

1. Shut down the instrument, see Section 7.1.
2. Open the instrument lid.
3. Clean each well hole in turn with a cotton swab moistened with 70% ethanol.
4. Allow the heating block to dry before closing the instrument lid.

7.5 Changing the fuses

The fuses of the PyroMark Q96 ID should be checked if the instrument power indicator is not illuminated, even though the power cable is connected to the mains power outlet and the standby switch is on.

Important: Ensure that the PyroMark Q96 ID is disconnected from the mains power before removing fuses from the instrument.

Have a qualified technician (e.g., facilities staff) ensure that the mains power outlet is live.

The following item is required:

- Small flat-head screwdriver

Procedure:

1. Turn off the standby switch at the front of the instrument and disconnect the mains power cable.
2. If necessary, carefully turn the instrument, so that the rear panel can be easily accessed.
3. Use a small screwdriver to release the securing lugs on each side of the fuse holder.
4. Pull out the fuse holder using your fingers. The fuse holder contains two fuses (type F 6.3 A H, 250 V). Check both fuses and replace as necessary.

   Note: Replace fuses only with fuses of the specified type and current rating.
5. Insert the fuse holder (it is keyed and can only be replaced in one way) and push it into place using your fingers.
6. Reposition the instrument, reconnect the power cable and turn the standby switch on.
7. If the instrument still does not work, please contact QIAGEN Technical Services.

7.6 Cleaning and testing the reagent cartridge

If the reagent cartridge is to be reused, it must be cleaned directly after use. It is recommended that the reagent cartridge is used a maximum of 20 times.

WARNING	**Sharp needles** [W7] Do not touch the sharp needles at the bottom of the reagent cartridge. Handle the needles with care. Small particles and fibers may obstruct the needles.

Note: Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

The following items are required:

- Powder-free gloves
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Clean, lint-free cloths

There are two possible cleaning procedures: A standard cleaning procedure (Section 7.6.1) and a cleaning procedure for clogged cartridges (Section 7.6.2). Ensure that a cleaned cartridge is usable by performing a cartridge function check (Section 7.6.3).

7.6.1 Standard cleaning procedure

Procedure:

1. Discard any remaining solutions.
2. Rinse the cartridge four times with high-purity water.
3. Spray the outside of the needles with high-purity water.
4. Fill the compartments to the top with high-purity water. Hold the cartridge over a sink or beaker while pressing firmly on top of each compartment with a finger (wear powder-free gloves). A jet of water should come straight out of the tip of the needle.
5. If a needle is blocked, wet the needle tip with a drop of high-purity water.

6. Press several droplets through each needle.
7. Discard the high-purity water.
8. Leave the cartridge to dry on its side, on a lint-free cloth. Do not apply the cap.

   Note: Ensure that the needles are dry on the outside before using them.
9. Store the cartridge in the PyroMark Q96 HS Tip Holder Box between uses.

7.6.2 Clogged cartridge cleaning procedure

If reagent solutions have been left in the cartridge, a more thorough cleaning process might be needed to clear the needles.

Procedure:

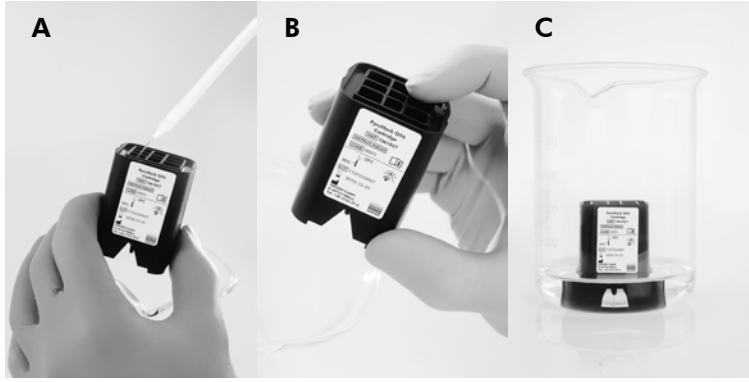
1. Discard any remaining solutions.
2. Rinse the cartridge several times with high-purity water.
3. Fill the compartments with high-purity water and immerse the cartridge in a beaker with high-purity water covering the needles. Leave the cartridge in the beaker for 1 hour.
4. Proceed with the standard cleaning procedure in section 7.6.1, beginning with step 4.

7.6.3 Cartridge function check

To check if a cartridge can be used for analysis, a simple function test can be performed.

1. Fill the compartments completely with high-purity water. Take care not to let the water run over the edge and wet the needles on the outside.
2. Press firmly on top of each compartment in turn with a finger (use powder-free gloves). A small jet of water should come out from the tip of each needle. If no jet is seen, fill the compartment with more water and try again.
3. Check that the jet of water is straight in the direction of the needle. If not, fill with water again and press firmly.

4. If the test fails, discard the cartridge.
5. After a cartridge has passed the function test, allow it to dry completely at ambient temperature (15–25°C) before using.



Cleaning the PyroMark Q96 Cartridge. **A** Filling the compartments with high-purity water. **B** Placing pressure on compartments. **C** Cartridge immersed in a beaker with high-purity water.

7.7 Cleaning the PyroMark Q96 Vacuum Workstation

WARNING	**Hazardous chemicals** [W4]
	The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

If the Vacuum Workstation needs to be cleaned to remove dust and spillage, follow the instructions below.

The following items are required:

- Powder-free gloves
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- A mild detergent (if necessary)
- Clean, lint-free cloths

Procedure:

1. Ensure that no vacuum is applied to the vacuum prep tool, i.e. the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Disconnect the vacuum pump from the mains power.
3. Clean the worktable and the tool, except for the filter probes, using a clean, lint-free cloth moistened with water or a mild detergent.
   Note: Do not touch the tips of the filter probes.
4. Wipe the worktable and the tool, except for the filter probes, dry using a clean, lint-free cloth.
5. Reconnect the vacuum pump to the mains power.

7.8 Testing and replacing the filter probes

7.8.1 Function test for filter probes

Before using the PyroMark Q96 Vacuum Workstation, check that the filter probes are working by performing the function test as follows:

1. Add 100 µl high-purity water to each well of a 96-well PCR plate.
2. Fill a trough with 70 ml high-purity water.
3. Start the vacuum pump.
4. Apply vacuum to the vacuum prep tool by opening the vacuum switch.
5. Lower the filter probes into the trough. Keep them in position for approximately 20 s. Ensure that the water is transferred to the waste container, i.e., that vacuum has been applied. If this has not happened, check the connections.
6. Lower the filter probes into the PCR plate and check that the water is aspirated evenly across all wells and that all wells are empty after a maximum of 10 s.
7. If the wells are not empty after 10 s, repeat the procedure from step 1. If the function test fails twice, replace the filter probes (see below).

7.8.2 Replacing filter probes

Each filter probe can be replaced individually. To ensure proper flow rate through the filter probes, all probes should be replaced after preparation of approximately 100 plates.

Note: Use gloves (powder-free) to avoid contaminating the filter probes.

The following items are required:

- Powder-free gloves
- Allen key
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- New filter probes (cat. no. 979010)

Procedure:

1. Ensure that no vacuum is applied to the Vacuum Prep Tool, i.e. the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Disconnect the vacuum pump from the mains power.
3. Remove the tool from the tubing.
4. Loosen the four screws using an Allen key.
5. Pull out the old filter probes.
6. Gently insert new filter probes without pressing on the filter tips.
7. Replace and fasten the four screws and reconnect the vacuum pump to the mains power.

7.9 Replacing the tubing

If the tubing is broken or distorted, replace it.

Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

The following items are required:

- New tubing (contact QIAGEN Technical Services)
- Beaker

Procedure:

1. Ensure that no vacuum is applied to the Vacuum Prep Tool, i.e. the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Disconnect the vacuum pump from the mains power.
3. Remove the broken tubing at one end and empty any liquid waste into an empty beaker.
4. Disconnect the other end of the tubing and discard the tubing and any liquid waste.
5. Cut the new vacuum tubing into three pieces and assemble it. Use the old tubing as a template for the size of the new tubing pieces. Ensure that the tubing is connected to the pump's vacuum fitting.
6. Reconnect the vacuum pump to the mains power.

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8 Troubleshooting

If you need to contact QIAGEN Technical Services about an error, note down the steps leading to the error and any information given in any dialog boxes. This will help the QIAGEN Instrument Service Specialist in solving the problem.

When calling QIAGEN Technical Service about errors, please have the following information ready:

- Instrument serial number, type, and version
- Date of last maintenance performed
- Error code (if applicable)
- Time point when the error occurred for the first time
- Frequency of error occurrence (i.e., intermittent or persistent error)
- Photo of error, if possible

Take the following action before contacting QIAGEN Technical Services.

1. Check important parameters in the "Run Overview" in the "Instrument Status" tab to assess if the system was working properly during the run.
2. Consult the Troubleshooting sections below.

8.1 Unexpected results

		Comments and suggestions
a)	No result in any wells of the plate	Check the reagent cartridge and ensure that it is correctly filled with reagents and/or nucleotides. Check that the needles are not damaged or obstructed and that the reagent cartridge cover is closed. Insufficient sample or incorrectly conditioned sample (e.g., too much dsDNA or salt present). Follow sample preparation instructions carefully (see Section 5.4). Check the PCR samples using a gel technique to confirm there is only one specific band.

		Comments and suggestions
b)	Results only in some wells of the plate	Insufficient sample amounts. Follow sample preparation instructions carefully (see Section 5.4). Wells marked in the software do not correspond to the sample placement in the PyroMark Q96 Plate Low. Check the plate was loaded according to the information under the "Sample" tab in the "Run Setup" dialog. Insufficient enzyme or substrate for all the wells. Check the reagent cartridge was filled according to the "Volume Information" under the "Run Info" tab.

8.2 Sequence errors

		Comments and suggestions
a)	Some peaks appear, but the sequence stops	Ensure that all nucleotide chambers in the reagent cartridge contain nucleotides. Check the reagent cartridge for blockages and rinse or replace it, as necessary. Check the sample and sample preparation. Contaminated samples will lead to an unusually high consumption of substrate at substrate addition, giving a high presequencing signal and no light produced at nucleotide dispensation.

	Comments and suggestions
b) Faulty or poor sequence	Contaminated or nonhomogeneous samples. Follow sample preparation instructions carefully, see Section 5.4.
	Reagents incorrectly stored or dissolved.
	Too much or too little template.
	Wrong sequencing primer added.
	Wrong sequence to analyze entered.
	Wrong dispensation order chosen.

8.3 Peak errors

	Comments and suggestions
a) Wide peaks	Assay-specific problem due to secondary structure.
	Contaminated or nonhomogeneous samples. Follow sample preparation instructions carefully, see Section 5.4.
	Reagents incorrectly stored or dissolved.
	Too much or too little template.
	Too much template DNA. Check amount of DNA template used.
	Reaction volume in wells was too high. Use lower volume.

	Comments and suggestions
b) Nonspecific peaks	Sequence signals from the self-annealed sequencing primer and/or biotinylated PCR primer and DNA template or nonspecific annealing of the sequencing primer.
	Run DNA template, sequencing primer, and biotinylated PCR primer in individual wells. If significant peaks appear, consider redesigning the PCR and/or sequencing primer(s).
	Contaminated nucleotides.
	Background of contaminating sequence from PCR. Consider redesigning sequencing primer.
	Contact QIAGEN Technical Services if this problem persists.
c) Low signal	Too little template.
	Reagents incorrectly stored or dissolved.
	Too much salt in the sample.
	Follow sample preparation instructions carefully, see Section 5.4.
	Dirty lens array. Clean the lens array, see Section 7.3.
d) High baseline noise	Contaminated nucleotides or background of contaminating sequence from PCR.
	Contact QIAGEN Technical Services if this persists.

		Comments and suggestions
e)	Occasional peaks between dispensations	Cross-talk or dispensation errors. Avoid placing assays with high signals close to assays with low signals. Check and replace the reagent cartridge, if necessary. Light leaking into the instrument. Do not place the instrument in strong sunlight. Contact QIAGEN Technical Services for camera calibration.

8.4 Instrument-related errors

		Comments and suggestions
a)	No contact between the operator's computer and the PyroMark Q96 ID	Check mains power supply to instrument and ensure the "Power" light is illuminated. Check the network cables are properly connected at the rear of the instrument and the wall outlets. If contact was broken during a run, a restore of the run may be necessary. No match between IP numbers for the instrument and the operator's computer or the IP number conflicts with other equipment on the network. Contact your system administrator.
b)	All lights on the PyroMark Q96 ID remain on 5 minutes after it has been switched on	Instrument software problem. Contact QIAGEN Technical Services.

		Comments and suggestions
c)	Heating block temperature control is not working correctly	Remove and reinsert the heating block, see Section 7.4. If there is liquid between the heating block and the instrument, clean the lens array, see Section 7.3. If the problem persists, the contact pins may need replacing. Contact QIAGEN Technical Services.

8.5 Vacuum workstation-related errors

		Comments and suggestions
a)	No vacuum is received	Turn off the vacuum pump and open the cap to the waste container to release any pressure. Close the cap and start the pump again. Empty the waste container if full. Ensure that the tubing is connected correctly and that there is no leakage.
b)	Vacuum lost during sample preparation	Ensure that the tubing is connected correctly and there is no leakage.
c)	Filter probes not working properly	Ensure filter probes are working properly; see Section 7.8.1.
d)	Liquid left in some wells in the immobilization plate	Replace the corresponding filter probes; see Section 7.8.2.
e)	White remains (Sepharose beads) in the immobilization plate	If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.

9 Glossary

Term	Description
Biotin	A molecule that can bind very strongly to streptavidin. PCR primers can be biotinylated to enable the resulting PCR product to bind to streptavidin-coated Sepharose beads.
Dispensation order	Defines the nucleotides and the order in which they should be dispensed in Pyrosequencing runs.
Drop off	A continual decrease in signal height normally seen in the Pyrogram.
Enzyme	A protein (or RNA) working as a catalyst, to enhance the speed of a biochemical reaction without altering it. In Pyrosequencing technology, a mixture of Klenow polymerase, sulfurylase, luciferase, and apyrase is used in the sequencing reaction.
Histogram	The theoretical representation of the expected Pyrosequencing peak pattern.
Homopolymer	A stretch of identical bases in DNA. In Pyrosequencing technology, a stretch of more than two identical bases is regarded as a homopolymer.
Instrument parameters	A method that describes physical settings for the instrument, such as mixer frequency, block temperature, and pulse time settings.

Term	Description
IUPAC	International Union of Pure and Applied Chemistry. An organization providing recommendations on organic and biochemical nomenclature, symbols, terminology, etc. IUPAC codes A = Adenine C = Cytosine G = Guanine T = Thymine R = Purine (A or G) Y = Pyrimidine (C or T) M = C or A K = T or G W = T or A S = C or G B = C, T, or G (not A) D = A, T, or G (not C) H = A, T, or C (not G) V = A, C, or G (not T) N = Any base (A, C, G, or T)
Out of phase	When one of the alleles is sequenced ahead of the other.
Polymorphism	Genetic variations, broadly encompassing any of the many types of variations in DNA sequences that are found within a given population.
Presequencing signal	As the substrate is dispensed into a well (indicated by an "S"), contaminants (such as ATP or PPi) will be converted to light. Too high a substrate peak indicates that high levels of a contaminant might be present in, for example, buffers.
Pyrogram	The graph resulting from a sequencing reaction performed using Pyrosequencing technology. Each incorporated nucleotide is shown as a peak in Pyrogram.

Term	Description
Reference peak	Nonvariable peaks, i.e., peaks that are not a part of an SNP site, are referred to as "reference peaks". Reference peaks are used in the analysis both as references when calculating the single peak height, and as internal controls when assessing the quality.
RLU	Relative Light Unit (entity used in Pyrosequencing to define peak heights in Pyrogram).
Sepharose beads	Streptavidin-coated beads can be used for preparation of biotinylated PCR products.
Sequence to analyze	A short part of a DNA sequence (in your sample), starting directly after the sequencing primer, which contains one or several variable positions to be analyzed using Pyrosequencing instrument platforms.
Sequencing primer	The sequencing primer is annealed to the template during the sample preparation. The 3'-end of the sequencing primer serves as the starting point for the extension by the polymerase.
Shift	Plus shift: A small proportion of the template sequences that incorporates more than one type of nucleotide at a time (if, for example, there are residues left from the dispensation before) and will be sequenced ahead of the rest of template sequences. Minus shift: A small proportion of the template sequences that fails to incorporate a nucleotide will be sequenced subsequent to the rest of template.
Signal-to-noise ratio	The ratio of the signal height and the noise height. An indication of the clarity of the data. The higher the ratio, the better the data.
Single nucleotide polymorphism (SNP)	SNPs involve the change of one DNA base to another. SNPs and point mutations are structurally identical, differing only in their frequency. Variations that occur in 1% or less of a population are considered point mutations, while those occurring in more than 1% are SNPs.

Term	Description
Streptavidin	A protein that can bind very strongly to biotin.
Substrate	A molecule acted upon by an enzyme. Pyrosequencing technology uses a mixture of the substrates Adenosine 5' phosphosulfate (APS) and Luciferin in the sequencing reaction.
Theoretical outcome	The possible genotypes for a specific polymorphic position are calculated on the basis of the sequence to analyze and the dispensation order.

Appendix A

Technical data

QIAGEN reserves the right to change specifications at any time.

Environmental conditions

Operating conditions

Power	100–120 V, 220–240 V; 50/60 Hz Power consumption: 280 VA max.
Overvoltage category	II
Air temperature	18–28°C (64–82°F)
Relative humidity	30–90% relative humidity (noncondensing)
Altitude	Up to 2000 m (6500 ft.)
Place of operation	For indoor use only Draft-free location, not close to window. Keep instrument out of direct sunlight
Pollution level	2
Environmental class	3K2 (IEC 60721-3-3)

Transportation conditions

Air temperature	–25°C to 60°C (–13°F to 140°F)
Relative humidity	Max. 75% (noncondensing)

Storage conditions

Air temperature 10°C to 40°C (50°F to 104°F)

Relative humidity Max. 75% (noncondensing)

Mechanical data and hardware features: PyroMark Q96 ID

Dimensions (closed)	Width: 520 mm (20.47 in.) Height: 490 mm (19.29 in.) Depth: 620 mm (24.41 in.)
Clearance space	Width: 540 mm (21.26 in.) Height: 570 mm (22.44 in.) Depth: 640 mm (25.20 in.)
Mass	52 kg (114.64 lb.)
Capacity	Up to 96 samples per run
Chemical resistance	Instrument covers: pH 4 to pH 9, common detergents, 70% ethanol Inside the instrument: 70% ethanol

PyroMark Q96 Software

Operating system	Microsoft Windows XP (English version) or Microsoft Windows 7, 32 bit (English version)
Processor	Intel Core Duo processor (2 GHz) or higher
RAM	1 GB
Free hard disk space	200 MB
Graphics card	Supporting the resolution of the monitor
Monitor	1280 x 800 pixels, True Color (32 bit) or higher
Pointer device	Mouse or similar
Interfaces	Ethernet ports (or external USB Ethernet adaptor).

Waste Electrical and Electronic Equipment (WEEE)

This section provides information about disposal of waste electrical and electronic equipment by users.

The crossed-out wheeled bin symbol (see below) indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to applicable local laws and regulations.

The separate collection and recycling of waste electrical and electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.

Recycling can be provided by QIAGEN upon request at additional cost. In the European Union in accordance with the specific WEEE recycling requirements and, where a replacement product is being supplied by QIAGEN, free recycling of its WEEE-marked electronic equipment is provided.

To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

FCC declaration

The ''United States Federal Communications Commission'' (USFCC) (in 47 CRF 15. 105) declared that the users of this product must be informed of the following facts and circumstances.

This device complies with part 15 of the FCC. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This Class B digital apparatus complies with Canadian ICES-003.

The following statement applies to the products covered in this manual, unless otherwise specified herein. The statement for other products will appear in the accompanying documentation.

Note: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/ TV technician for help.

QIAGEN's FCC Compliance Tests were conducted using QIAGEN-supported peripheral devices and shielded cables, such as those you receive with your system. Changes or modifications not expressly approved by QIAGEN could void the user's authority to operate the equipment.

Declaration of Conformity

Name and address of the company

QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
Germany

We herewith declare that the product

PyroMark® Q96 ID

meets all applicable requirements of the following European Directives

Low Voltage Directive (LVD)	**2006/95/EC**
Electromagnetic Compatibility Directive (EMC)	**2004/108/EC**

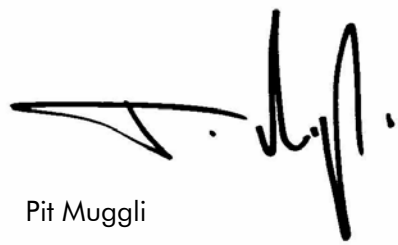
and the relevant harmonized standards

IEC 61010-1:2001
IEC 61010-2-081:2001/A1:2003
IEC 61326-1:2006

Accessories

PyroMark Q96 Vacuum Workstation

Hombrechtikon, March 4, 2011



Pit Muggli

Senior Director Quality and Compliance Automated Systems

CE

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Appendix B

Assay design and validation

Assay design

Pyrosequencing assays can be designed using the latest version of PyroMark Assay Design Software (ADSW). The program automatically generates primer sets that include both PCR and sequencing primers. Each primer set is given a quality score based on several parameters that are specific for Pyrosequencing analysis. Ensure you use the correct assay type in PyroMark ADSW.

PCR

For PCR amplification we recommend using the PyroMark PCR Kit (Cat. no. 978703) which is specifically optimized for Pyrosequencing analysis and enables highly specific and unbiased amplification of template DNA for various Pyrosequencing applications, such as mutation detection, SNP analysis, methylation analysis, and *de novo* sequencing. The convenient master mix format enables specific amplification of various starting materials such as genomic DNA from a variety of sources, as well as bisulfite converted DNA, using only one protocol.

PCR primers

One of the primers must be biotin-labeled to enable immobilization of the PCR amplicons to streptavidin-coated beads (Streptavidin Sepharose High Performance, GE Healthcare) for the preparation of a single-stranded DNA template. The orientation of the assay can either be forward or reverse. If designing primers with PyroMark ADSW, the primer that needs to be biotinylated is indicated.

The biotinylated primer should be purified by HPLC or an equivalent procedure since free biotin will compete with the biotinylated PCR product for binding sites on the streptavidin-coated Sepharose beads.

Amplicon length

The optimal amplicon length for Pyrosequencing assays is between 80 and 200 bp, although products up to 500 bp may work well. Amplicons for CpG assays should ideally be shorter than 200 bp.

Sequencing primer

Design sequencing primers using PyroMark ADSW. When designing an InDel assay, it is highly recommended that the sequencing primer is located a few bases before the variable position.

PCR setup

PCR reactions of 25 µl are set up using the PyroMark PCR Kit. Ensure that you follow the instructions provided in the *PyroMark PCR Handbook*.

Run the PCR at the optimal annealing temperature for 45 cycles. Using fewer cycles may produce insufficient yield of template DNA and interfere with subsequent Pyrosequencing reactions due to the presence of unused biotinylated primer.

The PCR product should give one strong band with minimal excess of primers when analyzed on an agarose gel.

Starting template

The yield and quality of PCR product is affected by both the quality and quantity of the nucleic acid starting template. This is particularly true for amplification of long regions from DNA that has been fragmented by bisulfite-treatment or extracted from paraffin-embedded material.

Quality of starting template

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, ethanol, EDTA, and other chemical solvents than single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR.

These include the QIAprep® system for rapid plasmid purification, the QIAamp® and DNeasy® systems for rapid purification of genomic DNA and viral nucleic acids, and the RNeasy® system for RNA preparation from a variety of sources. For more information about QIAprep, QIAamp, DNeasy, and RNeasy products, contact one of our Technical Service Departments (see back cover) or visit www.qiagen.com.

Quality of starting template when performing CpG assays

Critical parameters for a successful PCR using bisulfite-treated DNA templates include complete bisulfite conversion and DNA fragments that are long enough for PCR. EpiTect® Plus Bisulfite Kit provides a fast and reliable procedure for efficient bisulfite conversion and a unique DNA Protect Buffer prevents DNA fragmentation during the bisulfite conversion reaction. For more information about EpiTect products, contact one of our Technical Service Departments (see back cover or visit www.qiagen.com).

Quantity of starting template

The annealing efficiency of a primer to the template is an important factor in PCR. Owing to the thermodynamic nature of the reaction, the primer:template ratio strongly influences the specificity and efficiency of PCR and should be optimized empirically. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mispriming events.

PCR optimization

The PyroMark PCR Kit will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, we recommend using the 25 mM $MgCl_2$ provided in the kit.

The recommended annealing temperature is 60°C and 56°C for genomic DNA and bisulfite treated DNA, respectively, when using PyroMark ADSW 2.0.

Addition of Q-Solution® (provided with the PyroMark PCR Kit) may improve PCR yield and specificity for difficult templates that, for example, have a high degree of secondary structure or templates that are GC-rich.

For all PCR optimization tests, analyze 5 µl of a 25µl PCR on an agarose gel and aim for one strong specific band with minimal excess of primers.

Please refer to the *PyroMark PCR Handbook* for further troubleshooting.

Equal amplification of both alleles in AQ and CpG assays

Reliable results in quantification assays depend on equal amplification of both alleles and this must be carefully tested.

To ensure equal amplification in a CpG assay, unmethylated DNA can be mixed with increasing proportions of completely methylated DNA. We recommend using EpiTect Control DNAs (Cat. no. 59695), which provide bisulfite-treated completely methylated and unmethylated DNA in ready-to-use solutions. Regression analysis of the frequency of one allele measured in the PyroMark Q96 ID as a function of the input (expected) allele, should give an R^2 value greater than 0.9.

For an AQ assay, the allelic variants, including the variable position, can be mixed at different ratios similar to the procedure for a CpG assay. If the variable position in an AQ assay is a SNP, the easiest way to test for equal amplification is to compare the peak heights from a heterozygote. If the SNP is represented by single base incorporations, e.g., AAC/TGG, the two alleles (C and T peaks) should give peaks of equal height. An InDel heterozygote should give 50% deletion.

Sample preparation

Use 5–20 µl of a 25 µl PCR for immobilization of PCR amplicons to streptavidin-coated sepharose beads (Streptavidin Sepharose High Performance, GE Healthcare) according to the instructions in Section 5.4.3. This will be equivalent to approximately 1–4 picomoles of PCR product, depending on the PCR efficiency.

Note: When using the PyroMark PCR Kit, 20 µl of the PCR product gives satisfactory Pyrosequencing results in most cases. This volume should be adjusted to achieve single peak heights of at least 20 RLU in the Pyrogram.

Pyrosequencing analysis

Use default settings in the software for all assay setups if not otherwise stated.

Assay setup

AQ and CpG assays

When creating an AQ or CpG assay, the sequence to analyze should contain a sufficient number of bases to generate at least five nonvariable reference peaks. For InDels, a few reference peaks should be included before the variable position.

If the sequencing primer is placed immediately adjacent to the position to analyze, include part of the sequence following the variable position in the "Sequence to Analyze" text box. Ensure that the last base in the text box is not part of the variable position.

Blank dispensations are automatically generated by the software and serve as built-in quality controls for the assay. Never exclude the blank dispensations generated by the software as they function as excellent indicators of unspecific nucleotide incorporation. When manually generating a dispensation order, include an appropriate number of blank dispensations. If possible, start the dispensation order with

one blank dispensation and have at least the same number of blank dispensations as the number of variable positions.

Pay attention to tips and warnings indicated by the red icon and make suitable modifications when the icon appears.

Bisulfite treatment controls in CpG assays

When creating a CpG assay, inclusion of bisulfite treatment controls is recommended. Cytosines not followed by a Guanine, indicated as orange **T**s in PyroMark ADSW, should be fully converted to Thymine during bisulfite treatment and can therefore be used as controls for the reaction. When creating a CpG assay, the software indicates possible dispensations as controls for the bisulfite treatment reaction. The original sequence (before bisulfite treatment) can be entered in the assay and used to see if the suggested controls are **C**s converted to **T**s (read as **G**s and **A**s in a reverse assay) and suitable as controls, or not. The preferable controls are those dispensations that are located at the beginning of the sequence and/or represent single base incorporations.

SQA assays

Based on experience sequencing large numbers of templates, the dispensation order n(CTGA) gives, on average, the best *de novo* sequencing quality. Individual templates may, however, give better results with other dispensation orders.

SQA assays set up to differentiate multiple sequences should not be designed to distinguish variants based on accurate sequencing of homopolymers. In addition, it may be useful to have a few known bases at the beginning of the sequence, preferably single peaks. These can be used as reference peaks to aid the setting of the peak levels in difficult assays.

Ensure that the initial DNA sample is pure or that the assay is capable of specifically amplifying and/or sequencing only one target sequence in the sample. The assay may otherwise generate a mixed sequence that cannot be analyzed.

Pay attention to tips and warnings indicated by the red icon and make suitable modifications when the icon appears.

Validation of a new assay

Controls

All new assays have to be validated by the user. Use a reference DNA sample when testing a new assay and ensure that appropriate analysis parameters in the PyroMark Q96 Software are used. Interactions between primers or loops formed on single-stranded DNA can serve as priming sites for base incorporation by DNA polymerase. The following controls should be included when an assay is analyzed for the first time:

- PCR without template DNA. This will show if the primers interact to give a background signal in Pyrosequencing reactions.
- PCR with template DNA but with no sequencing primer. This will show if the template can loop back on itself and give a background signal in Pyrosequencing reactions.
- Sequencing primer without any PCR product. This will show if the sequencing primer can form duplexes or hairpins and give background signal in Pyrosequencing reactions.
- Biotinylated primer without any PCR product. This will show if the biotinylated primer can form duplexes or hairpins and give background signal in Pyrosequencing reactions.
- Sequencing primer and biotinylated primer together without PCR product. This will show if the sequencing primer and the biotinylated primer can form duplexes and give background signal in Pyrosequencing reactions.

Pyrograms from these controls should not show a peak after any nucleotide addition.

Quality assessment

The user will be warned if something in the assay may reduce the quality of the result given by the analysis software. The ultimate goal for a well-optimized assay is that all variable positions in an AQ or CpG assay, or the sequence in the quality control window of a SQA assay, have the quality assessment "Passed" when using default or more stringent analysis parameters. Such results will be shown as blue in the quality bar in the well after analysis. Results of lower quality are indicated as "Check" (yellow) or "Failed" (red) together with error messages.

Analysis results

For samples and positive controls, aim for:

- Sufficient signal intensities. Aim for a single peak height of at least 20 RLU
- No background in blank dispensations
- No background in variable positions (AQ and CpG)
- Expected reference sequence pattern (AQ and CpG)
- All positions (AQ and CpG) and quality control window (SQA) with quality assessment "Passed".

The quality assessments for AQ and CpG assays are based on the sequence context as well as the results in the analyzed positions. Deviations from this built-in quality control are shown as warnings in the "Well Information" area.

The analysis results for SQA assays are based on the appearance of the peaks in Pyrogram, related to peak height levels estimated by the software.

The inclusion of known bases in SQA assays can improve the estimation of peak height level.

Appendix C

Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications.

All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

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Appendix D

1 Informations de sécurité

Avant d'utiliser le PyroMark Q96 ID, il est impératif de lire attentivement ce manuel et de porter une attention particulière aux informations de sécurité. Afin de garantir un fonctionnement du système en toute sécurité et de maintenir le système en bon état de marche, il est impératif de suivre les instructions et les informations de sécurité fournies dans le présent manuel d'utilisation.

Les types d'informations de sécurité suivants sont fournis tout au long du manuel.

AVERTISSEMENT	Le terme AVERTISSEMENT signale des situations risquant d'entraîner des **accidents corporels** dont l'utilisateur, ou d'autres personnes, pourraient être victime. Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

ATTENTION	Le terme ATTENTION signale des situations risquant d'entraîner des **détériorations de l'appareil** ou de tout autre matériel. Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

Les conseils donnés dans ce manuel ont pour but de venir compléter les exigences de sécurité habituelles en vigueur dans le pays de l'utilisateur et non de s'y substituer.

1.1 Utilisation appropriée

AVERTISSEMENT/ ATTENTION	**Risque d'accident corporel et de détérioration du matériel** L'utilisation inappropriée du PyroMark Q96 ID peut entraîner des accidents corporels ou une détérioration de l'appareil. L'utilisation du PyroMark Q96 ID est réservée exclusivement au personnel qualifié ayant été convenablement formé. L'entretien du PyroMark Q96 ID doit être effectué uniquement par des spécialistes de l'entretien sur site QIAGEN.

Procéder à la maintenance comme décrit à la Section 7. QIAGEN facture les réparations rendues nécessaires suite à une maintenance inappropriée.

AVERTISSEMENT/ ATTENTION	**Risque d'accident corporel et de détérioration du matériel** L'appareil PyroMark Q96 ID est trop lourd pour être soulevé par une seule personne. Afin d'éviter tout accident corporel et toute détérioration du matériel, ne pas soulever l'appareil seul.

1.2 Sécurité électrique

Avant l'entretien, débrancher les cordons d'alimentation des prises de courant.

AVERTISSEMENT	**Danger électrique** Toute interruption du conducteur de protection (conducteur de terre/de masse) à l'intérieur ou à l'extérieur de l'appareil ou toute déconnexion de la borne du conducteur de protection est susceptible de rendre l'appareil dangereux. Toute interruption intentionnelle est interdite. **Tensions mortelles à l'intérieur de l'équipement** Lorsque l'équipement est relié à l'alimentation, les bornes peuvent être sous tension et l'ouverture de capots ou le retrait de pièces risque d'exposer des éléments sous tension.

Afin que le PyroMark Q96 ID fonctionne de manière satisfaisante et en toute sécurité, suivre les conseils suivants :

- Les cordons d'alimentation de l'appareil doivent être branchés sur des prises d'alimentation munies d'un conducteur de protection (terre/masse).
- Les fiches d'alimentation reliées au secteur doivent être facilement accessibles s'il est nécessaire de débrancher rapidement l'équipement du secteur.
- Utiliser uniquement les fiches et cordons d'alimentation fournis avec le système.
- Si vous remplacez les fusibles, ne les remplacer qu'avec des fusibles du même type et équivalent en voltage, comme indiqué sur le fusible

1.3 Produits chimiques

AVERTISSEMENT	**Produits chimiques dangereux** La solution de dénaturation utilisée avec le poste de travail sous vide contient de l'hydroxyde de sodium qui est irritant pour les yeux et la peau. Toujours porter des lunettes de protection, des gants et une blouse de laboratoire. La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que le lieu de travail environnant est sûr et que les opérateurs de l'appareil ne sont pas exposés à des niveaux dangereux de substances (chimiques ou biologiques) toxiques comme cela est défini dans les fiches de données de sécurité (FDS) ou dans les documents de l'OSHA *, de l'ACGIH † ou du COSHH ‡ applicables. Pour plus d'informations, voir le site www.qiagen.com/safety. L'évacuation des vapeurs et la mise au rebut des déchets doivent s'effectuer conformément à toutes les réglementations et lois nationales, régionales et locales relatives à la santé et à la sécurité.

* OSHA : Occupational Safety and Health Administration (États-Unis d'Amérique) (Administration pour la santé et la sécurité du travail).

† ACGIH : American Conference of Government Industrial Hygienists (États-Unis d'Amérique) (Conférence américaine des hygiénistes industriels gouvernementaux).

‡ COSHH : Control of Substances Hazardous to Health (Royaume-Uni) (Contrôle des substances dangereuses pour la santé).

1.4 Dangers mécaniques

Quand l'appareil PyroMark Q 96 ID est en marche, son couvercle doit rester fermé.

AVERTISSEMENT	**Pièces mobiles** Afin d'éviter le contact avec les pièces en mouvement lorsque l'appareil PyroMark Q96 ID est en marche, son couvercle doit rester fermé. Ne pas démonter les panneaux du capot. Ils ne renferment aucune pièce réparable par l'utilisateur. En cas de problème avec l'appareil PyroMark Q96 ID, contacter immédiatement les Services techniques de QIAGEN.

AVERTISSEMENT	**Risque de pincement et d'impact** L'unité de distribution, le couvercle de la chambre de réaction et l'instrument peuvent bouger à tout moment.

AVERTISSEMENT	**Aiguilles pointues** Ne pas toucher les aiguilles pointues situées au fond de la cartouche de réactifs. Manipuler les aiguilles avec précaution. De petites particules ou des fibres peuvent boucher les aiguilles.

1.5 Dangers liés à la chaleur

AVERTISSEMENT	**Surface chaude** Le bloc chauffant peut atteindre des temperatures jusqu'à 80°C (176°F). Eviter de le toucher quand il est chaud.

ATTENTION	**Risque de surchauffe** Afin de garantir une bonne ventilation, laisser un dégagement d'au moins 10 cm sur les côtés et à l'arrière de l'appareil PyroMark Q96 ID. Les fentes et les ouvertures qui assurent la ventilation de l'appareil ne doivent pas être obstruées.

1.6 Symboles sur le PyroMark Q96 ID

Symbole	Emplacement	Langue	Description
CE	Plaque signalétique à l'arrière de l'appareil	EN	Marquage CE
REF	Réactifs, solutions, cartouche, plaque	EN	Référence
	Réactifs, solutions, cartouche, plaque	EN	Limitations de température
	Plaque signalétique à l'arrière de l'appareil et de tous les autres produits	EN	Fabricant légal

Symbole	Emplacement	Langue	Description
	À l'intérieur de l'appareil	EN	Avertissement, se reporter au manuel d'utilisation.
	Type de plaque à l'arrière de l'instrument et de la boite	EN	Numéro de série
	Plaque signalétique à l'arrière de l'appareil	EN	Symbole DEEE
	Plaque signalétique à l'arrière de l'appareil	EN	Label FCC de la Federal Communications Commission des États-Unis
	Plaque signalétique à l'arrière de l'appareil	EN	Label C-Tick pour l'Australie (identifiant du fournisseur N17965)
	Plaque signalétique à l'arrière de l'appareil	EN	Label RoHS pour la Chine (restriction de l'utilisation de certaines substances dangereuses dans l'équipement électrique et électronique)
	Plaque signalétique à l'arrière de l'appareil	EN	Conforme aux normes de sécurité des États Unis et du Canada

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Appendix E

1 Sicherheitshinweise

Vor der Inbetriebnahme des PyroMark Q96 ID sollten Sie dieses Handbuch sorgfältig durchlesen – beachten Sie insbesondere die Sicherheitshinweise. Die Anweisungen und Sicherheitsinformationen in diesem Handbuch müssen vom Anwender befolgt werden, um einen sicheren Betrieb des PyroMark Q96 ID zu gewährleisten und das Gerät in einem sicheren Zustand zu erhalten.

In diesem Handbuch werden die folgenden beiden Kategorien von Sicherheitshinweisen verwendet:

WARNUNG	Der Begriff „WARNUNG“ (“WARNING”) weist Sie auf Situationen hin, in denen eine **Verletzungsgefahr** für Sie selbst oder andere Personen besteht. Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

ACHTUNG	Der Begriff „ACHTUNG“ (“CAUTION”) weist Sie auf Situationen hin, in denen das **Gerät oder andere Geräte beschädigt** werden könnten. Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

Die in diesem Handbuch enthaltenen Hinweise stellen eine Ergänzung und keinen Ersatz der üblichen Sicherheits-anforderungen dar, die im jeweiligen Land gelten.

1.1 Sachgemäße Handhabung

WARNUNG/ ACHTUNG	**Verletzungsgefahr und Beschädigung des Geräts** Die unsachgemäße Bedienung des PyroMark Q96 ID kann zu einer Verletzung des Benutzers oder zur Beschädigung des Geräts führen. Die Bedienung des PyroMark Q96 ID darf nur durch qualifiziertes, entsprechend geschultes Personal erfolgen. Die Instandhaltung des PyroMark Q96 ID darf nur durch einen Servicespezialisten des QIAGEN Außendiensts durchgeführt werden.

Führen Sie alle Wartungsarbeiten gemäß den Anweisungen in Abschnitt 7 dieses Handbuchs durch. QIAGEN® stellt alle Reparaturen in Rechnung, die nachweislich auf eine inkorrekte Wartung zurückzuführen sind.

WARNUNG/ ACHTUNG	**Verletzungsgefahr und Beschädigung des Geräts** Der PyroMark Q96 ID ist sehr schwer und sollte nicht von einer Person angehoben werden. Heben Sie das Gerät nicht allein an, um eine Verletzung und/oder Beschädigung des Geräts zu vermeiden.

1.2 Schutz vor Stromschlag

Ziehen Sie die Netzanschlusskabel aus den Steckdosen, bevor Sie Wartungsarbeiten am Gerät vornehmen.

WARNUNG	**Gefahr durch Stromschlag** Jede Unterbrechung des Schutzleiters (Erdungs- bzw. Masseleiter) im Gerät oder außerhalb des Geräts und jede Abtrennung des Schutzleiters am Anschluss der Netzleitung erhöht die Gefahr eines Stromschlags. Eine absichtliche Unterbrechung der Schutzleiter-verbindung ist verboten. **Gefährliche Spannung im Gerät** Wenn das Gerät an die Stromversorgung angeschlossen ist, sind die Anschlussstellen spannungsführend. Durch das Öffnen der Abdeckungen oder das Entfernen von Gehäuseteilen können spannungsführende Komponenten freigelegt werden.

Um einen zufriedenstellenden und sicheren Betrieb des PyroMark Q96 ID zu gewährleisten, befolgen Sie bitte die nachstehenden Hinweise:

- Die Geräte-Netzkabel müssen an Wechselstrom-Steckdosen mit Schutzleiter (Erdungs-/Masseleiter) angeschlossen werden.
- Sorgen Sie dafür, dass die Netzstecker jederzeit frei zugänglich sind, falls das Gerät einmal schnell vom Stromnetz getrennt werden muss.
- Benutzen Sie nur Netzanschlüsse und -kabel, die mit dem Gerät geliefert werden.
- Beim Austausch der Netzsicherung ersetzen Sie diese nur durch eine desselben Typs und der Stromstärke, die auf dem Etikett/Typenschild angegeben ist.

1.3 Chemikalien

WARNUNG	**Gefährliche Chemikalien**
	Die Denaturierungslösung, die zusammen mit der Vakuum-Arbeitsstation verwendet wird, enthält Natriumhydroxid, das auf Augen und Haut reizend wirkt. Tragen Sie immer eine Schutzbrille, Laborhandschuhe und einen Laborkittel. Die verantwortliche Person (z. B. der Laborleiter) muss alle erforderlichen Vorsichtsmaßnahmen treffen, um sicherzustellen, dass die unmittelbare Umgebung des Arbeitsplatzes sicher ist. Auch dürfen die Grenzwerte in Bezug auf infektiöse Erreger, die in den entsprechenden Sicherheitsdatenblättern (SDS) oder den Vorschriften der OSHA*, ACGIH† oder COSHH‡ festgelegt sind, nicht überschritten werden. Weitere Informationen finden Sie unter www.qiagen.com/safety. Beim Betrieb eines Abzugs und bei der Entsorgung von Abfallstoffen müssen alle Bestimmungen und Gesetze zu Gesundheitsschutz und Sicherheit am Arbeitsplatz auf übernationaler, nationaler und regionaler Ebene eingehalten werden.

* OSHA: Occupational Safety and Health Administration (Vereinigte Staaten von Amerika)

† ACGIH: American Conference of Government Industrial Hygienists (Vereinigte Staaten von Amerika)

‡ COSHH: Control of Substances Hazardous to Health (Vereinigtes Königreich)

1.4 Gefahren durch mechanische Teile

Der Deckel des PyroMark Q96 ID muss während des Betriebs geschlossen sein.

WARNUNG	**Sich bewegende Geräteteile** Um einen Kontakt mit sich bewegenden Teilen beim Betrieb des PyroMark Q96 ID zu vermeiden, darf das Gerät nur mit geschlossenem Deckel betrieben werden. Entfernen Sie nicht die Abdeckplatten; im Geräteinneren befinden sich keine Bauteile, die vom Anwender gewartet werden müssen. Setzen Sie sich umgehend mit dem Technischen Service von QIAGEN in Verbindung, falls einmal ein Problem mit dem PyroMark Q96 ID auftreten sollte.

WARNUNG	**Quetsch- und Stoßgefahr** Die Dispensiereinheit, der Prozesskammer-Deckel und/oder Teile des Automationsmoduls könnten jederzeit in Bewegung gesetzt werden.

WARNUNG	**Scharfe Nadeln** Berühren Sie nicht die scharfen Nadeln am Boden der Reagenzienkartusche. Gehen Sie vorsichtig mit den Nadeln um. Kleine Partikel oder Fasern können die Nadeln verstopfen.

1.5 Überhitzungsgefahr

WARNUNG	**Heiße Oberflächen** [W8] Der externe Heizblock kann Temperaturen von bis zu 80 °C erreichen. Berühren Sie diese Teile nicht, wenn sie aufgeheizt sind.

ACHTUNG	**Überhitzungsgefahr** Vergewissern Sie sich, dass ein Mindestabstand von 10 cm zwischen Seitenwänden und Rückseite des PyroMark Q96 ID und der Wand eingehalten wird, damit eine ausreichende Belüftung des Geräts gewährleistet ist. Die Lüftungsschlitze und Öffnungen, die für die Be- und Entlüftung des PyroMark Q96 ID bestimmt sind, dürfen nicht verdeckt werden.

1.6 Symbole auf dem PyroMark Q96 ID

Symbol	Fundstelle	Sprache	Beschreibung
	Typenschild auf der Geräterückseite	EN	CE-Kennzeichnung
	Reagenzien, Lösungen, Kartusche, Platte	EN	Katalognummer
	Reagenzien, Lösungen, Kartusche, Platte	EN	Zulässiger Temperaturbereich
	Typenschild auf der Rückseite des Geräts und allen anderen Produkten	EN	Hersteller i. S. d. Gesetzes
	Im Geräteinneren	EN	Warnhinweis; lesen Sie das Handbuch

Symbol	Fundstelle	Sprache	Beschreibung
	Typenschild auf Geräterückseite und Verpackungskarton	EN	Seriennummer
	Typenschild auf der Geräterückseite	EN	WEEE-Markierung
	Typenschild auf der Geräterückseite	EN	FCC-Kennzeichen der Federal Communications Commission der Vereinigten Staaten
	Typenschild auf der Geräterückseite	EN	C-Tick-Mark-Zeichen für Australien (Herstellerkennung: N17965)
	Typenschild auf der Geräterückseite	EN	Markierung gemäß RoHS-Richtlinie für China (Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten)
	Typenschild auf der Geräterückseite	EN	Entspricht US-amerikanischen und kanadischen Sicherheitsstandards

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